

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

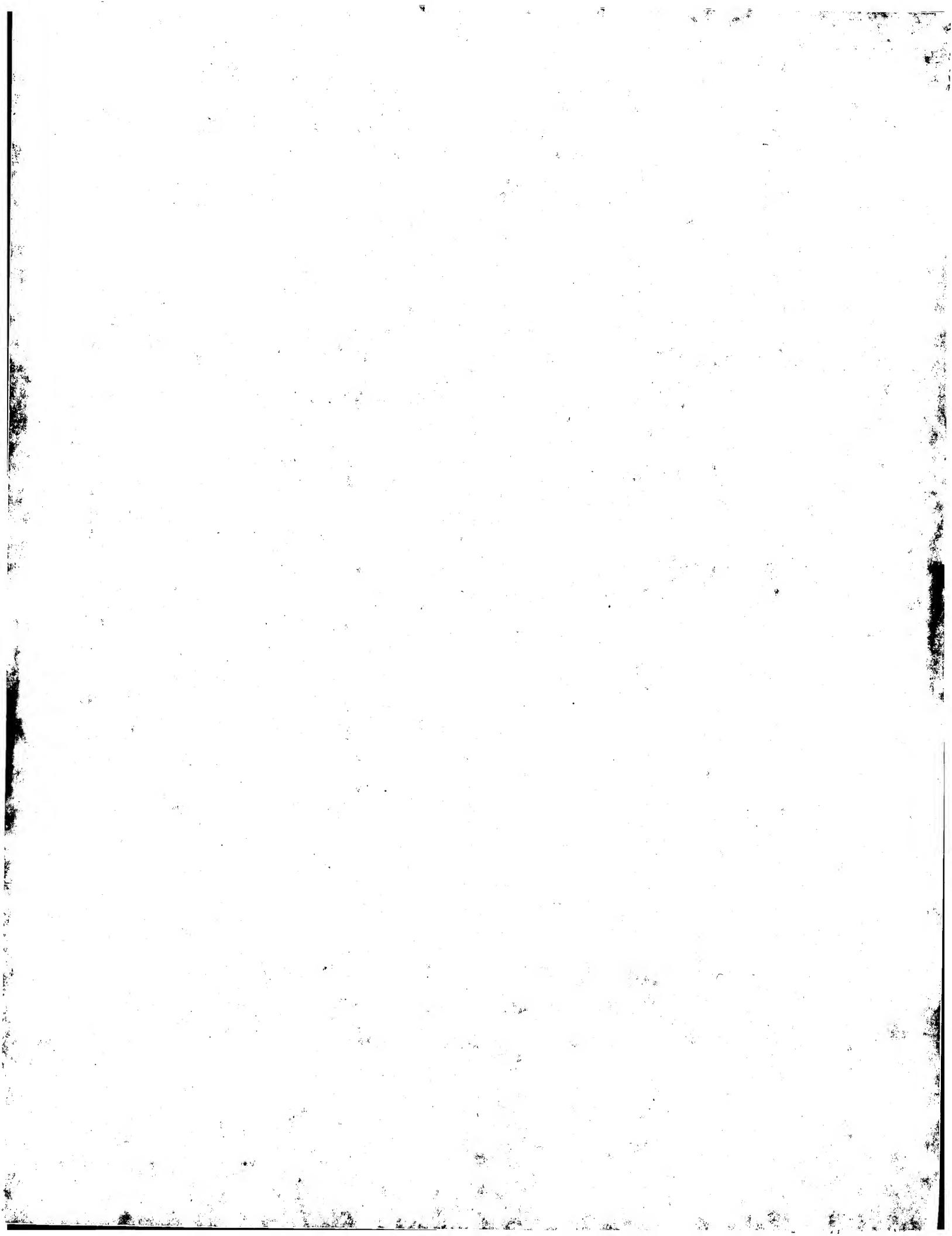
Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**



25. Okt.

99972928-1 B9901

PCT

WORLD INTELLECTUAL PROPERTY ORGANISATION
International Bureau

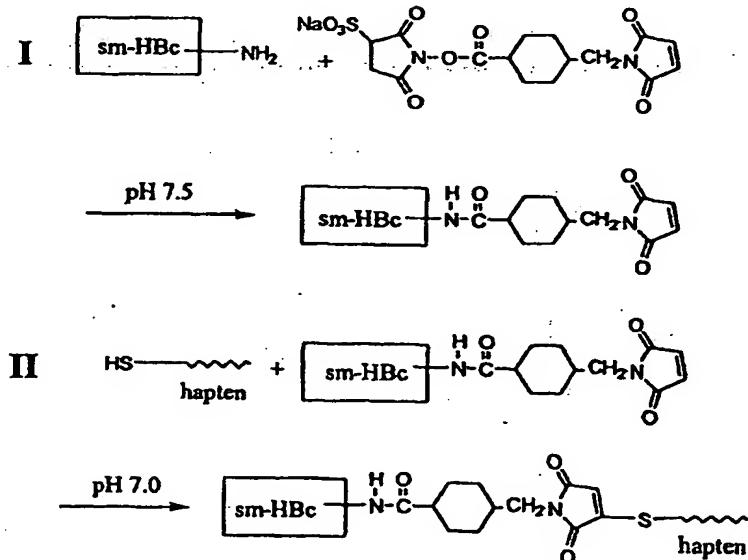
25. Okt. 2001

Document AMS
Appl. No. 10/622,064

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6: A61K 39/00, 39/38, 39/29, C07K 7/00, 15/00	A1	(11) International Publication Number: WO 99/40934 (43) International Publication Date: 19 August 1999 (19.08.99)
(21) International Application Number: PCT/US99/03055		(81) Designated States: AL, AU, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, HU, ID, IL, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SD, SG, SI, SK, SL, TR, TT, UA, UZ, VN, YU, ARIPO-patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
(22) International Filing Date: 11 February 1999 (11.02.99)		
(30) Priority Data: 60/074,537 12 February 1998 (12.02.98) US		
(71) Applicant: IMMUNE COMPLEX, CORPORATION [US/US]; 3347 Industrial Court, San Diego, CA 92121 (US).		Published With international search report.
(72) Inventor: BIRKETT, Ashley, J.; Apartment 3208, 878 Stevens Avenue, Solana Beach, CA 92075 (US).		
(74) Agents: GAMSON, Edward, P. et al.; Welsh & Katz, Ltd., 22nd floor, 120 South Riverside Plaza, Chicago, IL 60606 (US).		

(54) Title: STRATEGICALLY MODIFIED HEPATITIS B CORE PROTEINS AND THEIR DERIVATIVES



(57) Abstract

A strategically modified hepatitis B core protein is described, where an insert is provided, preferably in an immunodominant region of the nucleocapsid protein, containing a chemically reactive amino acid residue. The modified hepatitis B core protein or its aggregated nucleocapsid protein particles can be pendently linked to a hapten to form a modified nucleocapsid conjugate. Such a conjugate is useful in the preparation of vaccines or antibodies. The modified hepatitis B core protein can also be modified to include a T cell epitope.

1997-2000

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

-1-

STRATEGICALLY MODIFIED HEPATITIS B CORE
PROTEINS AND THEIR DERIVATIVES

Description

5

Technical Field

The present invention relates to the intersection of the fields of immunology and protein engineering, and particularly to carrier proteins, and more particularly to a hepadnavirus nucleocapsid protein strategically modified with an inserted chemically-reactive amino acid residue, a pendently-linked hapten conjugate of that hepadnavirus nucleocapsid protein and to an immunogenic particle comprised of those conjugates.

Background of the Invention

It is known that antibodies can be raised to a small molecule by using a large immunogenic protein molecule as a carrier. The small molecule that derives enhanced immunogenicity by being conjugated to the carrier is called a hapten. The phenomenon of a relatively large molecule potentiating the immunogenicity of a small molecular entity to which it is attached is known in the art as the "carrier effect".

The portion of an immunogen recognized by the helper T cell (Th cell) is the T cell determinant or epitope. The portion of an immunogen that is bound by antibody is the B cell determinant or epitope. Carrier effects can be defined as immunity to one determinant, the "helper" or T (T_h) cell

WO 99/40934

-2-

determinant, of a multideterminant immunogen enhancing the immune response to another determinant, the B cell determinant.

It is now well established that most 5 immunogens require T cell help to induce B cells to produce antibodies. Thus, T_h cells, by recognizing helper determinants on the immunogen help B cells to make antibody against the immunogen.

The antigenic determinants recognized by T 10 helper cells (T_h) and B cells must be associated to form a single molecular entity, but they do not have to be covalently linked. See, Russel et al., *Nature*, 280:147 (1979), Lamb et al., *J. Immunol.*, 129:1465 (1982), Scherle et al., *J. Exp. Med.*, 164:1114 (1986) 15 and Lake et al., *Cold Spring Harbor Symp. Quant. Biol.*, 41:589 (1976). Some immunogens do not require T cell help to induce antibody formation, these are T-independent antigens.

A pathogen-related protein can be 20 immunologically mimicked by the production of a synthetic polypeptide whose sequence corresponds to that of a determinant of the pathogen. Such polypeptide immunogens are reported by Sutcliffe et al., *Nature*, 287:801 (1980), and Lerner et al., *Proc. Natl. Acad. Sci. USA*, 78:3403 (1981).

Gerin et al., *Proc. Natl. Acad. Sci. USA*, 80:2365 (1983), showed limited protection of chimpanzees from hepatitis B virus upon immunization with carrier-bound synthetic polypeptides having 30 amino acid residue sequences that correspond to the sequence of a determinant portion of HBsAg; in

WO 99/40934

PCT/US99/03055

-3-

particular, residues 110-137 of the "S" (surface) region. However, the carrier protein used in those studies was keyhole limpet hemocyanin (KLH), a T cell-dependent carrier that is not fit for use in medical applications to humans because it is a source of irritation that leads to severe inflammation.

T cell-stimulating carrier proteins capable of enhancing the immunogenicity of haptens that do not produce unacceptable side effects in human subjects are often immunogenic natural proteins. For example, tetanus toxoid (TT) has been frequently used when a carrier suitable for human administration was needed. However, the use of tetanus toxoid as a carrier was restricted due to problems with dosage limitations and risk of sensitization to the toxoid itself. In addition, an epitope-specific suppression of response to the carried hapten can occur in individuals already immunized against tetanus.

The hepatitis B surface protein has been proposed as a carrier for heterologous epitopes. Delpeyroux et al., *Science*, 233:472-475 (1986), reported the use of the HBV surface protein (S protein) as a carrier for a poliovirus polypeptide hapten. Those investigators constructed a recombinant deoxyribonucleic acid (DNA) protein expression vehicle that produces a fusion protein, designated HBsPolioAg, capable of forming particles closely resembling authentic 22-nanometer HBsAg particles. HBsPolioAg consists of HBV S protein having an 11 amino acid residue sequence insert

corresponding to amino acids 93-103 of capsid protein
VPI of poliovirus type 1 (Mahoney strain).

Hepadnavirus nucleocapsid proteins have been used as hapten carriers. Heterologous 5 immunogenic peptide sequences inserted internally in the hepatitis B core, expressed as fusion particles, elicited very high immune responses in immunized animals in the absence of adjuvants. B.E. Clarke et al. *Vaccines* 91:313-318 (1991); F. Schödel et al. *J. Virol.* 66(1):106-114 (1992). U.S. Patent Nos. 10 4,818,527, 4,882,145, and 5,143,726, the disclosures of which are incorporated herein by reference, describe the use of the carrier effect with hepatitis, B virus nucleocapsid protein to enhance the 15 immunogenicity of an operatively linked polypeptide hapten. Those patents describe the linking of a polypeptide hapten to hepatitis B virus nucleocapsid protein through an amino acid residue side chain that occurs naturally in the hepatitis B nucleocapsid 20 protein sequence.

Hepadnavirus nucleocapsid proteins are fairly well studied. SEQ ID NOS:1 and 2 are the DNA and amino acid sequences of the human hepatitis B core protein (HBc), subtype ayw, as described in U.S. 25 Patent Nos. 4,818,527, 4,882,145, and 5,143,726. Other hepadnavirus nucleocapsid protein sequences are also known in the art, see e.g. SEQ ID NOS: 3-13.

There are reasons to select hepadnavirus 30 nucleocapsid proteins as a carrier over other carriers used in the art, such as keyhole limpet hemocyanin (KLH), BCG, tetanus toxoid and diphtheria

-5-

toxoid. KLH, BCG, tetanus toxoid and diphtheria toxoid are non-particulate, whereas hepadnavirus nucleocapsid proteins tend to aggregate into "particles". HBC particles tend to have a higher immunogenicity than hepatitis B surface antigen (HBsAg) particles. D.R. Milich et al., *Science*, 234:1398-1401 (1986). HBC is both a T cell-independent and a T cell-dependent immunogen. *Id.* HBC is one of the most immunogenic proteins known.

Almost all hepatitis B-infected people develop a powerful immune response to core. J.H. Hoofnagle, *Semin. Liver Dis.*, 1(1):7-14 (1981). HBC can provide universal responsiveness, irrespective of genetic background. *Id.* HBC directly activates T cells.

HBC elicits strong Th cell responses. HBC is efficiently processed and presented by antigen-presenting cells. Due to the inherently high immunogenicity of HBC, complex adjuvants are typically not required, for example, the common and inexpensive alum is sufficient.

The family hepadnaviridae is a family of enveloped animal viruses with a core of DNA that cause hepatitis B in humans. The hepadnaviridae are not responsible for human hepatitis A (a single-stranded RNA enterovirus), human hepatitis C (Flaviridae family of single stranded RNA virus), or human hepatitis D (a closed circular negative-sense RNA satellite virus, "delta virus", that requires hepatitis B virus for replication). The hepadnaviridae family includes hepatitis viruses of other species, e.g. woodchuck, duck, ground squirrel,

WO 99/0934

PCT/US99/03055

-6-

and heron, in addition to human and simian hepatitis

B. Hepatitis B (HB) used hereinafter refers to the family hepadnaviridae, unless the discussion is referring to a specific example.

5 Hepatitis B core protein monomers self-assemble into stable aggregates known as hepatitis B core protein particles (HBc particles). For example, human HBc particles are 27 nanometers (nm) in diameter. Conway et al., *Nature*, 386:91-94 (1997),
10 describe the structure of human HBc particles at 9 Ångstrom resolution, as determined from cryo-electron micrographs. Bottcher et al., *Nature*, 386:88-91 (1997), describe the polypeptide fold for the human HBc monomers, and provide an approximate numbering
15 scheme for the amino acid residues at which alpha helical regions and their linking loop regions form. Bottcher et al. propose a loop from about residues 78 to 82 of the hepatitis B core protein.

Using synthetic peptides and monoclonal
20 antibodies, the immunodominant loop region of HBc was mapped to about amino acid residues 75 to 83. G. Colucci et al., *J. Immunol.*, 141:4376-4380 (1988). Two immunodominant linear epitopes were reported by other workers at amino acid residues 75 to 85 and 130 to 140. Salfeld et al. *J. Virol.* 63:798 (1989).

25 Insertion mutants of the hepatitis B core protein still are able to form core particles when foreign epitopes are cloned into the immunodominant loop region of HBc. P. Pumpens et al., *Intervirology*, 38:63-74 (1995). The HBc fusion

WO 99/40934

PCT/US99/03055

-7-

proteins form particles in prokaryotic and eukaryotic expression systems. *Id.*

The ability to use a protein as a carrier for a pendently-linked hapten depends upon several factors that have been studied with respect to HBC. Chemically-reactive amino acid side chains, such as lysine (K), aspartic acid (D), glutamic acid (E), and reduced cysteine residues (C), provide functional groups that can be useful for modifying polypeptides.

The hepatitis B core protein sequence has several chemically-reactive amino acid side chains in the native sequence. Core has three primary amino groups, one at the amino terminus, and two lysine residues (K5 and K96), along with four cysteine residues (C48, C61, C107 and C183). There are several carboxylic acid groups, D (2, 4, 22, 29, 32, 78, 83) and E (8, 14, 40, 43, 46, 64, 77, 113, 117, 145, 179) and the carboxy terminus.

However, the native, unmodified hepatitis B core protein particle does not exhibit appreciable chemical reactivity of the amino acid side chains in the native sequence. The chemical reactivity of an amino acid side chain in a protein depends upon the nature of the amino acid side chain, and its environment in the folded protein.

As is discussed in detail hereinafter, it has now been found that the problem of low reactivity of the amino acid side chains in native hepatitis B nucleocapsid protein can be overcome by inserting a chemically-reactive amino acid side chain into the HBC protein sequence. A strategically modified

WO 99/40934

PCT/US99/03055

-8-

hepadnavirus core protein particle of the present invention exhibits substantially enhanced reactivity toward derivitization of HBc particles with chemically linked haptens, and provides enhanced immunogenicity to those linked hapten.

5 These modified HBc proteins and their pendentily-linked hapten conjugate derivatives are discussed in the disclosure that follows.

10 Brief Summary of the Invention

The present invention relates to a strategically modified hepatitis B core (HBc) protein that is linked to a pendent hapten through chemically-reactive amino acid residue inserted into the HBc sequence. The contemplated strategic modification of HBc is an insert mutation of the HBc protein. A contemplated insert is 1 to about 40 amino acid residues in length, preferably 1 to 10 amino acid residues in length, and includes a chemically-reactive amino acid residue.

20 The insert is provided to the region corresponding to amino acid residues about 50 to about 100 of the hepatitis B core protein sequence shown in SEQ ID NO:2. The preferred region of insertion corresponds to the hepatitis B core protein immunodominant loop region at about amino acid residue 70 to about 90, more preferably the loop tip region at about amino acid 78 to about 82 and most preferably at amino acid 78 to amino acid 80.

30 Such an introduced chemically-reactive amino acid residue is characterized in that it has a

WO 99/40934

PCT/US99/03055

-9-

chemically-reactive side chain that provides a chemical group for pendently linking the strategically modified HBC to the hapten. Typically, the chemically-reactive amino acid residue is a 5 lysine, cysteine, or histidine residue or a carboxyl-containing residue such as aspartic acid or glutamic acid, preferably lysine or a carboxyl-containing residue, and most preferably lysine.

The hapten bonded to the chemically-reactive amino acid residue is any compound of interest for generating an immune response, and is typically a B cell determinant. Preferably, the hapten is a polypeptide hapten, a carbohydrate hapten, or a non-peptidal/non-saccharidal (chemical) 15 hapten. In one embodiment of the invention, the hapten is a pathogen-related hapten, such as a B cell determinant of a pathogen.

In another embodiment of a strategically modified hepatitis B core protein conjugate, the 20 strategically modified hepatitis B core protein also has a T cell stimulating amino acid residue sequence operatively linked to the carboxy terminus of the hepatitis B core amino acid sequence. Preferably, both the hapten and the T cell stimulating amino acid residue sequence are pathogen-related, most 25 preferably, both are related to (from) the same pathogen.

In the above embodiment of the invention, the response to a B cell epitope is boosted by also 30 providing the T helper (T_h) cell determinant. In this preferred embodiment of the invention, such a T_h cell

WO 99/40934

PCT/US99/03055

-10-

determinant is from the same pathogen as the B cell determinant hapten that is pendently linked to the strategically modified hepatitis B core protein in order to provide pathogen-specific T cell memory in addition to the hepatitis B core protein antigen-specific T cell memory.

A strategically modified hepatitis B core protein conjugate contains a hapten that is pendently linked to a strategically modified hepatitis B core protein. Looked at differently, a before-described strategically modified hepatitis B core protein can be considered to have three peptide-linked domains, I, II and III (numbered consecutively from the amino terminus). Domain I comprises a sequence that corresponds to residues numbered about 10 to 50 of the amino acid sequence of hepatitis B core protein of SEQ ID NO:2, and preferably corresponds to residues numbered 1 to 50 of that sequence. Domain II is bonded to the carboxy terminal residue of Domain I. Domain II corresponds to residues numbered 50 to 100 of the amino acid sequence of hepatitis B core protein of SEQ ID NO:2. Domain III comprises a sequence that is bonded to the carboxy terminal residue of Domain II. Domain III corresponds to residues numbered 100 to about 140 of the amino acid sequence of hepatitis B core protein, and preferably corresponds to residues numbered 100 to about 149 of that sequence.

In an embodiment of the invention discussed before, a strategically modified hepatitis B core protein additionally has a Domain IV exogenous to HBC

-11-

that is peptide-bonded to the carboxy terminal residue of Domain III to provide a fusion protein. Domain IV is a T cell epitope.

A strategically modified hepatitis B core 5 protein particle of the invention is made of assembled hepatitis B core protein where a plurality of the subunits are strategically modified hepatitis B core protein subunits. Also contemplated is a particle comprised of a mixture of strategically 10 modified hepatitis B core protein subunits and other hepatitis B core protein subunits.

A contemplated strategically modified hepatitis B core protein particle conjugate is comprised of assembled hepatitis B core protein 15 subunits where a plurality of the subunits are strategically modified hepatitis B core protein subunits. In this embodiment, a hapten is pendently linked to a hepatitis B core protein subunit. Preferably, the hapten is pathogen-related. As 20 above, a T cell stimulating amino acid residue sequence can be peptide-bonded to the carboxy terminal residue of the sequence corresponding to hepatitis B core protein. Preferably that pathogen-related T cell determinant is related to the same 25 pathogen as the pathogen-related hapten.

A strategically modified hepatitis B core protein particle conjugate of the invention has pendentily-linked hapten. In a contemplated embodiment of the particle conjugate, the particle is 30 made up of a mixture of strategically modified hepatitis B core protein subunits having pendentily-

-12-

linked haptens, and other hepatitis B core protein subunits. In one embodiment, about 0.1 to about 0.5 of the strategically modified hepatitis B core protein subunits are pendently linked to a hapten.

5 Also contemplated is a particle conjugate that is made up of a mixture of strategically modified hepatitis B core protein subunits and other hepatitis B core protein subunits.

A before-described strategically modified hepatitis B core protein of the invention includes a peptide insert containing a chemically-reactive amino acid residue. That insert can be, but is typically not itself a separate B cell antigenic determinant, although some B cell immunogenicity of the insert can be exhibited merely because of the placement of the insert into the HBc protein or particle. Such an insert can be and in some embodiments is a T cell epitope. Placement of an insert into the HBc loop region greatly diminishes the HBc immunogenicity and antigenicity of the resulting molecule.

An inoculum of the invention comprises an immunogenic amount of the strategically modified hepatitis B core protein conjugate of the invention. When the pendently-linked hapten is a pathogen-related hapten, the inoculum can be used as a vaccine to protect a mammal treated with the inoculum from that pathogen. Thus, in one embodiment of the invention, a strategically modified hepatitis B core protein conjugate is used as a vaccine to provide protection against the pathogen from which the hapten is derived. More preferably, the inoculum is

-13-

comprised of strategically modified hepatitis B core protein particle conjugate as the immunogen.

The present invention has several benefits and advantages.

5 One benefit of a contemplated modified HBC protein is that the protein can be derivitized while in the aggregated form of HBC particles.

An advantage of the invention is that the modified HBC protein displays appreciably enhanced
10 chemical reactivity toward derivitization, as compared to use of the N-terminal primary amine, for example.

Another benefit of a contemplated modified HBC protein is that the chemistry of derivitization
15 of such side chains is well-studied, straightforward and relatively predictable.

Another advantage of a contemplated modified HBC protein is that it enhances the immunologic response to the conjugated hapten with
20 which it is derivitized.

Yet another benefit of a contemplated modified HBC protein is that it is unlikely to produce undesirable immunologic side effects in humans.

25 Still further benefits and advantages of the invention will be apparent to the skilled worker from the discussion that follows.

Brief Description of the Drawings

30 In the figure forming a portion of this disclosure Scheme 1 illustrates a reaction sequence

-14-

for pendently linking a hapten to a strategically modified hepatitis B core protein (sm-HBc) particle using sulpho-succinimidyl 4-(N-maleimidomethyl) cyclohexane 1-carboxylate (sulpho-SMCC). The sm-HBc particle is depicted as a box having (for purposes of clarity of the figure) a single pendent amino group.

Detailed Description of the Invention

10

A. Definitions

The term "antibody" refers to a molecule that is a member of a family of glycosylated proteins, called immunoglobulins, which can specifically combine with an antigen.

15 The word "antigen" has been used historically to designate an entity that is bound by an antibody, and also to designate the entity that induces the production of the antibody. More current usage limits the meaning of antigen to that entity bound by an antibody, whereas the word "immunogen" is used for the entity that induces antibody production. Where an entity discussed herein is both immunogenic and antigenic, reference to it as either an immunogen or antigen will typically be made according to its intended utility.

20 The word "hapten" is used to describe molecules that are capable of stimulating an immune response (e.g., production of antibody) when chemically coupled to a protein carrier. The word is often used for small nonantigenic molecules in the

WO 99/40934

PCT/US99/03055

-15-

art, but herein, it merely refers to the molecule that is to be pendently linked to the carrier protein, even if it is antigenic or not small.

"Antigenic determinant" refers to the 5 actual structural portion of the antigen that is immunologically bound by an antibody combining site or T cell receptor. The term is also used interchangeably with "epitope".

The noun "conjugate" as used herein refers 10 to a molecule formed from a hapten pendently linked through an amino acid residue side chain to a carrier.

The term "conservative substitution" as used herein denotes that one amino acid residue has 15 been replaced by another, biologically similar residue. Examples of conservative substitutions include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another such as between arginine and lysine, between glutamic and aspartic acids or between glutamine and asparagine and the like. The term "conservative substitution" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid 20 in place of an unsubstituted parent amino acid provided that antibodies raised to such a polypeptide also immunoreact with the corresponding polypeptide having the unsubstituted amino acid.

The term "corresponds" in its various 30 grammatical forms as used in relation to peptide sequences means the peptide sequence described plus

25-10-2061

-16-

or minus up to three amino acid residues at either or both of the amino- and carboxy-termini and containing only conservative substitutions in particular amino acid residues along the polypeptide sequence.

5 "Epitope" refers to that portion of a molecule that is specifically bound by a T cell antigen receptor or an antibody combining site.

"Epitope" and "determinant" are used interchangeably.

10 As used herein, the term "fusion protein" designates at least two amino acid residue sequences not normally found linked together in nature operatively linked together end-to-end (head-to-tail) by a peptide bond between their respective terminal amino acid residues.

15 The phrase "hepatitis B" as used here refers in its broadest context to any member of the family hepadnaviridae, a family of enveloped DNA-containing animal viruses that can cause hepatitis B in human.

20 The phrase "HBc" as used here refers to T cell stimulating proteins having an amino acid residue sequence that corresponds to an amino acid residue sequence encoded by the hepatitis B virus (HBV) nucleocapsid protein gene. An exemplary well-known naturally occurring protein encoded by the human HBV nucleocapsid gene is the "core" protein, subtype ayw, having the biological sequences of SEQ ID NOs: 1 and 2. Galibert, et al., *Nature* 281:646 (1979). HBeAg protein, the precursor to HBc, includes the sequence of the hepatitis B core protein and a "pre-core" sequence at the amino terminus

-17-

thereof, as shown in SEQ ID NOS: 8 and 9 in the case of a ground squirrel hepatitis B nucleocapsid gene. The core protein sequence begins at amino acid position 31 therein, thus corresponding to amino acid 5 residue number 1 of SEQ ID NO:2. The sequences for other hepatitis B core proteins are known in the art. Human hepatitis B virus core protein subtype adr is provided in SEQ ID NOS: 3 and 4, and subtype adw is provided in SEQ ID NOS: 5 and 6. Ono et al., *Nucl. Acids Res.* 11:1747 (1983). Sequences are also provided for woodchuck hepatitis B core protein at SEQ ID NO:7 [Schödel et al., *Adv. Viral Oncol.* 8:73-102 (1989)], ground squirrel at SEQ ID NOS:8 and 9, heron at SEQ ID NOS:10 and 11, and duck at SEQ ID 10 NOS:12 and 13. For clarity, the amino acid numbering system shown in SEQ ID NOS:1 and 2 with respect to human hepatitis B core protein subtype ayw is used as a benchmark herein. Other HBC sequences can be aligned with that sequence using standard biological 15 sequence alignment programs and protocols to determine the amino acid residues that "correspond to the hepatitis B core protein sequence of SEQ ID NO:2", see e.g. Schodel et al., *Adv. Viral Oncol.* 8:73-102 (1989).

20 If reference is made to a polypeptide portion of any of the above described naturally occurring HBV nucleocapsid gene encoded proteins, that reference is explicit, either by stating, for example, that a T cell stimulating portion of the 25 particular protein is referred to or by explicitly designating the particular portion of the sequence,

-18-

as by indication of the included amino acid residue positions.

The term "immunoreact" in its various forms means specific binding between an antigen as a ligand 5 and a molecule containing an antibody combining site such as a Fab portion of a whole antibody.

The phrase "operatively linked" as used herein means that the linkage does not interfere with the ability of either of the linked groups to 10 function as described; e.g., to function as a T or B cell determinant.

The phrase "pendently linked" refers to a single linkage, either direct or via a bridge, from a HBC protein to another molecule at other than the 15 amino or carboxy termini. The phrase is used herein to describe the linkage between a hapten and a chemically-reactive amino acid side chain of a strategically modified hepatitis B core protein.

"Macromolecular assembly" refers to a non-covalently bonded aggregate of protein subunits. 20 Typically in this invention, the protein subunit is a strategically modified hepatitis B core protein monomer. As described in more detail hereinafter, those core protein monomers usually self-assemble 25 into spherical "core particles" having either 90 or 120 core protein dimers (a total of 180 or 240 core protein subunits). A spherical core particle is an example of a macromolecular assembly.

The phrase "pathogen-related" as used 30 herein designates a B cell or T cell immunogen that is capable of inducing the production of antibodies

that immunoreact with a pathogen in native form.

Exemplary pathogen-related B cell and T cell immunogens are illustrated hereinafter.

The words "polypeptide" and "peptide" as used interchangeably throughout the specification and designate a linear series of amino acid residues connected one to the other by peptide bonds between the alpha-amino and carboxy groups of adjacent amino acids. Polypeptides can be variety of lengths, either in their neutral (uncharged) forms or in forms which are salts, and either free of modifications such as glycosylation, side chain oxidation, or phosphorylation or containing these modifications. It is well understood in the art that amino acid residue sequences contain acidic and basic groups, and that the particular ionization state exhibited by the peptide is dependent on the pH of the surrounding medium when the protein is in solution, or that of the medium from which it was obtained if the protein is in solid form. Also included in the definition are proteins modified by additional substituents attached to the amino acid side chains, such as glycosyl units, lipids, or inorganic ions such as phosphates, as well as modifications relating to chemical conversions of the chains, such as oxidation of sulfhydryl groups. Thus, "polypeptide" or its equivalent terms is intended to include the appropriate amino acid residue sequence referenced, subject to those of the foregoing modifications which do not destroy its functionality. A peptide or polypeptide used as a hapten typically contains fewer

WO 99/40934

PCT/US99/03055

-20-

than 70 amino acid residues, and more typically contains a linear chain of about 5 to about 40 amino acid residues, and more preferably about 10 to about 25 residues. It is noted that a contemplated 5 polypeptide haptен can be longer than 70 residues, but such a polypeptide is shorter than the naturally occurring protein that shares its sequence.

The word "protein" designates a polypeptide having about 70 or more amino acid residues, and is a 10 naturally occurring entity.

The words "secrete" and "produce" are often used interchangeably in the art as to cells from which antibody molecules are obtained. Cells that produce antibodies may, however, not secrete those 15 molecules into their environment. Herein, the antibody molecules are secreted and are obtained from the blood stream (humoral antibody). Nevertheless, antibodies are generally referred to as being "produced" in keeping with the phrase utilized in the 20 art.

All amino acid residues identified herein are in the natural or L-configuration. In keeping with standard polypeptide nomenclature, [J. Biol. Chem., 243, 3557-59 (1969)], abbreviations for amino 25 acid residues are as shown in the following Table of Correspondence, Table 1.

Table 1.

**TABLE OF CORRESPONDENCE
SYMBOL**

1-Letter	3-Letter	AMINO ACID
Y	Tyr	L-tyrosine
G	Gly	glycine
F	Phe	L-phenylalanine
M	Met	L-methionine
A	Ala	L-alanine
S	Ser	L-serine
I	Ile	L-isoleucine
L	Leu	L-leucine
T	Thr	L-threonine
V	Val	L-valine
P	Pro	L-proline
K	Lys	L-lysine
H	His	L-histidine
Q	Gln	L-glutamine
E	Glu	L-glutamic acid
Z	Glx	L-glutamic acid or L-glutamine
W	Trp	L-tryptophan
R	Arg	L-arginine
D	Asp	L-aspartic acid
N	Asn	L-asparagine
B	Asx	L-aspartic acid or L-asparagine
C	Cys	L-cysteine

5 B. Strategically Modified Hepatitis B Core Protein

The present invention contemplates a strategically modified hepadnaviridae core ("HBc") protein that has an inserted chemically reactive amino acid residue for pendently linking with haptens such as polypeptides and carbohydrates. The strategic modification of the invention is the

WO 99/40934

PCT/US99/03055

-22-

insertion of 1 to about 40 amino acid residues including a chemically-reactive amino acid residue into the hepatitis B core protein sequence in the region corresponding to amino acid residues 50 to 100 of the HBc sequence of SEQ ID NO:2. Such an introduced chemically-reactive amino acid residue has a side-chain that provides a functional group for independently linking a hapten to the strategically modified carrier.

Hepadnaviridae have a nucleocapsid, or core, surrounded by a lipid envelope containing surface proteins. The nucleocapsid is a generally spherical aggregate of core proteins ("core antigen", HBcAg) dimers. In vitro, the hepatitis B core protein self-assembles into "particles", spherical shells of icosahedral symmetry made up of 90 or 120 hepatitis B core protein dimers, thus 180 or 240 protein subunits. The particles are about 280 or 310 Ångstroms in diameter, respectively. B. Bottcher et al., *Nature*, 386:88-91 (1997); J.F. Conway et al. *Nature* 386:91-94 (1997).

A contemplated strategically modified hepatitis B core protein also forms a macromolecular assembly. Such a particle can be present in the form of 180 or 240 protein subunits, although it does not have to be such a 90 or 120 dimer.

Hybrid core proteins with exogenous amino acid residues inserted in the region near amino acid residue 80 are reported to assemble into regular shells, even with inserts as large as 46 amino acids in length. A.I. Brown et al., *Vaccine*, 9:595-601



25-10-2003

WO 99/40934

PCT/US99/03055

-23-

(1991); F. Schödel et al., J. Virol., 66:106-114
(1992).

The hepadnaviridae core protein sequence used as a benchmark sequence herein is that of the 5 human hepatitis B core protein, subtype ayw, shown in SEQ ID NOS:1 and 2. Other subtypes of the human hepatitis B virus are known. SEQ ID Nos: 3 and 4 are human HBc, subtype adr, and SEQ ID NOS:5 and 6 are HBc subtype adw. The sequences of various animal 10 hepatitis core proteins are also published. The biological sequence of duck hepatitis core protein is disclosed herein as SEQ ID NO:12 and 13; a portion of the ground squirrel hepatitis nucleocapsid gene is at SEQ ID NO:8 and 9; woodchuck hepatitis core is at SEQ 15 ID NO:7 and heron hepatitis core at SEQ ID NOS:10 and 11. Exemplary animal hepatitis B core proteins are aligned with human hepatitis B core protein by F. Schödel et al., Adv. Viral Oncology 8:73-102 (1989).

20 i. Strategic Modification of the Core Protein

The present invention contemplates a strategically modified hepatitis B core protein conjugate that comprises a hapten that is pendently linked to a strategically modified hepatitis B core 25 protein (HBc). The strategically modified hepatitis B core protein itself comprises an amino acid sequence corresponding to the hepatitis B core protein amino acid sequence of SEQ ID NO:2 including the amino acid residues numbered about 10 to about 30 140 of that sequence. That HBc amino acid residue sequence additionally contains an exogenous amino

WO 99/40934

PCT/US99/03055

-24-

acid residue insert in the region corresponding to amino acid residues numbered about 50 to about 100 from the HBC amino terminus, wherein the exogenous insert (i) is 1 to about 40 amino acid residues in length, and (ii) contains a chemically-reactive amino acid residue. The hapten is pendently linked to the strategically modified HBC protein by means of a chemically-reactive amino acid residue present in the insert.

10 It is preferred that residues 1 through 10 of SEQ ID NO:2 be present in the strategically modified HBC protein molecule. It is further

15 preferred when any residue is absent or deleted from position 1 to 10 that those residues be deleted in sequence and that the remaining residues be present in sequence. Thus, if a five residue deletion were contemplated, the deleted residues would be numbered 1-5, leaving residues 6 through the desired HBC carboxy terminus present, plus the insert.

20 It is similarly preferred that residues numbered about position 140 through 149 of SEQ ID NO:2 be present in a strategically modified HBC protein molecule. As noted elsewhere herein, the region of HBC numbered 150 through the carboxy

25 terminus contains a plurality of arginine residues. Those residues bind nucleic acids on purification of HBC particles after expression, and the sequence containing those residues is preferably omitted from a strategically modified HBC protein molecule. As 30 was the case with the residues of positions 1 through 10, it is preferred that residues between about

-25-

position 140 and 149 be present and correspondingly absent in a sequential manner. Thus, where the carboxy terminal residue corresponds to the residue of position 146, the residues of positions 141-145 5 are also present and those of 147-149 are absent.

Most preferably, a contemplated HBC sequence is that shown in SEQ ID NO:2 from position 1 through position 149, plus the sequence of the insert.

The insert can be placed within the HBC 10 sequence in the region of positions numbered about 50 through about 100, as already noted. Preferably, the insert is present in the region corresponding to amino acid residues numbered about 70 to about 90.

More preferably, the insert is present in the region 15 corresponding to amino acid residues numbered 78 to 82. Most preferably, the insert is located in the region corresponding to residues numbered 78 through 80.

A strategically modified hepatitis B core 20 protein of the invention has from 1 to about 40 amino acid residues inserted. Preferably, the insert is 1 to 10 amino acid residues in length. The insert contains a chemically-reactive amino acid residue. The insertion of more than one chemically-reactive 25 amino acid residue is also contemplated.

It is contemplated that restriction endonuclease sites be provided in the gene construct for the strategically modified hepatitis B protein near the desired insert region. The nucleotides of 30 the restriction endonuclease site will be translated into amino acids upon expression, and that effect has

WO 99/40934

PCT/US99/03055

-26-

some bearing on the choice of endonuclease. Several restriction endonucleases are commercially available (e.g. from Promega Corp., Madison, Wisconsin) and their recognition site sequences and cleavage sites 5 well known in the art. Example 1 describes such a construct for a strategically modified hepatitis B core protein.

In one preferred embodiment, the insert is a single residue that is added as the chemically-reactive residue. In other preferred embodiments, 10 the use of restriction enzymes and their recognition sequences causes about three to about five residues to be inserted, including the desired chemically-reactive residue.

An insert containing a chemically-reactive amino acid residue is inserted into the native hepatitis B core protein at a position corresponding to an amino acid residue position from about 50 to 15 about 100. The preferred region of insertion into the hepatitis B core protein is in the immunodominant loop region (about amino acid residue 70 to about 20 90), more preferably in the loop region that corresponds to the native hepatitis B core protein position from about amino acid 78 to about 82. Most 25 preferably, the insert is placed at residues numbered 78 to 80 of SEQ ID NO:2.

As used herein when it is said that the insert is "at a position" it is meant that the amino terminus of the insert is peptide bonded to the carboxy terminus of the corresponding amino acid 30 residue of the hepatitis B core protein sequence

85702003

WO 99/40934

PCT/US99/03055

-27-

having that amino acid residue number. In other words, the insert immediately follows the residue at that stated position.

Insertion can be effected by generally utilized methods in the art. Genetic manipulation, by a PCR-based method is illustrated in Examples 1 and 5. In addition, oligonucleotide-mediated site directed mutagenesis as discussed in J.Sambrook et al. Molecular Cloning: a laboratory manual, 2nd ed., 10 Cold Spring Harbor Laboratory Press, 15.51-ff. (1989) can be used to add one codon by hybridizing a desired DNA sequence with a template that adds a codon for single residue, followed by filling in the remaining nucleic acid sequence. Dawson et al., Science 15 266:776-779 (1994), describe a method of linking polypeptide chains at their peptide backbone, so that a fusion could be built up of peptide fragments.

The chemically-reactive amino acid residue can be at any position within the insert. Preferably, the chemically-reactive amino acid residue is in a position that corresponds to amino acid residue numbered 70 to 90 of the native (wild type) core, and most preferably at residue position 78 to 82. For example, when a 10 amino acid residue long insert is inserted at a position corresponding to native core protein residue 73, then the chemically-reactive amino acid residue is preferably at position 5 to 9 of the insert. When a 30 amino acid residue long insert is inserted a position corresponding to native core protein residue 58, then

WO 99/40934

PCT/US99/03055

-28-

the chemically-reactive amino acid residue is preferably at position 22 to 24 of the insert.

An introduced chemically-reactive amino acid residue has a chemically-reactive side chain 5 that provides a functional group for derivitizing the strategically modified HBC (i.e. conjugating a hapten to the modified HBC). Useful side chain functional groups include epsilon-amino groups, beta-or gamma-carboxyl groups, thiol (-SH) groups and aromatic 10 rings (e.g. tyrosine and histidine). The chemically-reactive amino acid residue is typically a lysine, cysteine, or histidine residue or a carboxyl-containing residue such as aspartic acid or glutamic acid. Lysine is a particularly preferred chemically-reactive amino acid residue. 15

It is noted that the amino acid residue sequence of the hepatitis B core protein encoded by and shown in SEQ ID NOS:1 and 2, respectively, has two consecutive endogenous carboxyl-containing 20 residues, existing glutamic (glu, E) and aspartic (asp, D) acids, at positions 77 and 78. However, the present invention contemplates the introduction of at least one additional, exogenous chemically-reactive 25 amino acid residue. European Patent No. 385610 reports that unsatisfactory results were achieved in attempts to chemically couple polypeptide haptens to HBC particles. It is noted that those coupling attempts were directed toward amino groups and not carboxyl groups of the amino acid side chains. 30 In addition of the use of an individual chemically-reactive amino acid residue in the insert

WO 99/40934

PCT/US99/03055

-29-

such as aspartic acid or lysine, substantially any sequence of the desired length that contains a chemically-reactive amino acid residue can be used. Exemplary inserts of greater than a single residue 5 include the B cell HRV-2 VP2 epitope discussed in B.E. Clarke et al. *Vaccines* 91:313-318 (1991), the HBsAg Pre-S(1)27-53 sequence discussed in F. Schödel et al. *J. Virol.* 66(1):106-114 (1992) and the HBsAg Pre-S(2)133-143 sequence discussed in F. Schödel et 10 al. *Vaccines* 90:193-198 (1990). An appropriate T cell epitope discussed hereinafter as a hapten can also be used as an insert. Exemplary sequences include those of SEQ ID NOS: 28, 32, 33, 34, 47, 48, 49, 50 and 55.

15

ii. Additional Modification of the Core Protein

It is also contemplated that a hepatitis B core protein strategically modified as described above has other modifications. The contemplated 20 modifications of the strategically modified core include the nature of the insert containing the chemically-reactive amino acid residue, truncation of the amino terminus, truncation of the carboxy terminus, fusion at the carboxy terminus, pendent 25 linking to the carboxy-terminal region.

The insert containing the chemically-reactive amino acid residue to which the hapten is conjugated can have a use in addition to providing the chemically-reactive amino acid residue. It is 30 contemplated that an insert containing a chemically-reactive amino acid residue is a T cell stimulating

WO 99/40934

PCT/US99/03055

-30-

amino acid sequence. Such a T cell stimulating amino acid sequence is preferably a T cell epitope from the same source as the B cell epitope that will be the conjugated antigen, e.g. both from *Mycobacterium tuberculosis*. Exemplary epitopes are discussed hereinafter.

An insert containing a chemically-reactive amino acid residue can also be chosen in order to confer additional desirable properties, such as stability-enhancing or solubility-enhancing properties.

A strategically modified hepatitis B core protein can be chemically modified by methods well known in the art. Numerous such techniques are disclosed in Roger L. Lundblad, Techniques in Protein Modification, CRC Press (Ann Arbor, Michigan: 1994). Such chemical modifications are made to enhance or diminish properties, for example, a lysine amino group can be blocked to change the isoelectric point of the protein, causing it to separate differently on a chromatographic ion exchange resin.

It is also contemplated that the carboxy terminus of the core protein sequence be truncated, preferably down to about amino acid residue position 140. The arginine-rich sequence present beginning at residue 150 of SEQ ID NO:2 binds to nucleic acids and can hinder the purification and handling of the expressed core protein. In SEQ ID NO:2, the arginine-rich stretches begin at position 150. A preferred strategically modified HBC protein has a carboxy terminal valine (V) residue of residue 149.

-31-

iii. Making Strategically-Modified Core Protein

The strategic modification of the hepatitis B core protein is typically made by known processes in the art on the DNA level, for example by inserting the codons corresponding to the amino acids to be inserted. The engineered gene is then expressed in a convenient system known in the art, for example in a viral culture in infected immortalized cells.

Methods for producing HBcAg proteins in general and the pre-core, core and HBeAg proteins in particular, are well known in the art. The same methods readily adapted to the isolation of the modified core protein particles of the invention. In addition, HBcAg and HBeAg can be produced by a variety of well known recombinant DNA techniques. See, for example, U.S. Pat. No. 4,356,270 to Itakura and 4,563,423 to Murray et al., respectively. Those recombinant DNA techniques can be easily adapted to produce modified core particles of the invention. The modified core proteins can be conjugated with hapten before or after particle formation, preferably after core particle formation and purification.

C. Modified Hepatitis B Core Protein Conjugate

Any hapten against which antibody production is desired can be linked to a strategically modified hepatitis B core protein to form an immunogenic strategically modified hepatitis B core protein conjugate of this invention. The hapten of interest typically is a B cell determinant.

WO 99/40934

PCT/US99/03055

-32-

The hapt n can be a polypeptide, a carbohydrate (saccharide), or a non-polypeptide, non-carbohydrate chemical such as 2,4-dinitrobenzene.

Methods for operatively linking individual haptens to a protein or polypeptide through an amino acid residue side chain of the protein or polypeptide to form a pendently-linked immunogenic conjugate, e.g., a branched-chain polypeptide polymer, are well known in the art. Those methods include linking through one or more types of functional groups on various side chains and result in the carrier protein polypeptide backbone being pendently linked--covalently linked (coupled) to the hapten but separated by at least one side chain.

Methods for linking carrier proteins to haptens using each of the above functional groups are described in Erlanger, *Method of Enzymology*, 70:85 (1980), Aurameas, et al., *Scand. J. Immunol.*, Vol. 8, Suppl. 7, 7-23 (1978) and U.S. Pat. No. 4,493,795 to Nestor et al. In addition, a site-directed coupling reaction, as described in Rodwell et al., *Biotech.*, 3, 889-894 (1985) can be carried out so that the biological activity of the polypeptides is not substantially diminished.

Furthermore, as is well known in the art, both the HBcAg protein and a polypeptide hapten can be used in their native form or their functional group content can be modified by succinylation of lysine residues or reaction with cysteine-thiolactone. A sulfhydryl group can also be incorporated into either carrier protein or conjugate



25-10-2001

WO 99/40934

PCT/US99/03055

-33-

by reaction of amino functions with 2-iminothiolane or the N-hydroxysuccinimide ester of 3-(3-dithiopyridyl)propionate.

5 The HBC protein or hapten can also be modified to incorporate a spacer arm, such as hexamethylene diamine or other bifunctional molecules of similar size, to facilitate the pendent linking.

10 *Polypeptide hapten.* Methods for covalent bonding of a polypeptide hapten are extremely varied and are well known by workers skilled in the immunological arts. For example, following U.S. Patent No. 4,818,527, m-maleimidobenzoyl-N-hydroxysuccinimide ester (ICN Biochemicals, Inc.) or succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC, Pierce) is reacted with a strategically modified hepatitis B core protein to form an activated carrier. That activated carrier is then reacted with a polypeptide that either contains a terminal cysteine or to which an additional amino-20 or carboxy-terminal cysteine residue has been added to form a covalently bonded strategically modified hepatitis B core protein conjugate. As an alternative example, the amino group of a polypeptide hapten can be first reacted with N-succinimidyl 3-(2-pyridylthio)propionate (SPDP, Pharmacia), and that thiol-containing polypeptide can be reacted with the activated carrier after reduction. Of course, the sulfur-containing moiety and double bond-containing Michael acceptor can be reversed. These reactions 25 are described in the supplier's literature, and also in Kitagawa, et al., J. Biochem., 79:233 (1976) and 30

-34-

in Lachmann et al., in 1986 Synthetic Peptides as Antigens, (Ciba Foundation Symposium 119), pp. 25-40 (Wiley, Chichester: 1986).

U.S. Patent No. 4,767,842 teaches several modes of covalent attachment between a carrier and polypeptide that are useful here. In one method, tolylene diisocyanate is reacted with the carrier in a dioxane-buffer solvent at zero degrees C to form an activated carrier. A polypeptide hapten is thereafter admixed and reacted with the activated carrier to form the covalently bonded strategically modified hepatitis B core protein conjugate.

Particularly useful are a large number of heterobifunctional agents that form a disulfide link at one functional group end and a peptide link at the other, including N-succidimidyl-3-(2-pyridyldithio) propionate (SPDP). This reagent creates a disulfide linkage between itself and a thiol in either the strategically modified hepatitis B core protein or the hapten, for example a cysteine residue in a polypeptide hapten, and an amide linkage on the coupling partner, for example the amino on a lysine or other free amino group in the carrier protein. A variety of such disulfide/amide forming agents are known. See for example *Immun. Rev.* (1982) 62:185. Other bifunctional coupling agents form a thioether rather than a disulfide linkage. Many of these thioether-forming agents are commercially available and include reactive esters of 6-maleimidocaproic acid, 2-bromoacetic acid, 2-iodoacetic acid, 4-(N-maleimido-methyl)cyclohexane-1-carboxylic acid and

PCT/WO 99/40934

PCT/US99/03055

-35-

the like. The carboxyl groups can be activated by combining them with succinimide or 1-hydroxy-2-nitro-4-sulfonic acid, sodium salt. The particularly preferred coupling agent for the method of this invention is succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) obtained from Pierce Company, Rockford, Ill. The foregoing list is not meant to be exhaustive, and modifications of the named compounds can clearly be used, e.g., the sulpho-SMCC depicted in the figure.

A polypeptide hapten can be obtained in a number of ways well known in the art. Usual peptide synthesis techniques can be readily utilized. For example, recombinant and PCR-based techniques to produce longer peptides are useful. Because the desired sequences are usually relatively short, solid phase chemical synthesis is useful.

As discussed below, DNA sequences that encode a variety of polypeptide haptens are known in the art. The coding sequence for peptides of the length contemplated herein can easily be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci et al., *J. Am. Chem. Soc.* 103:3185 (1981). Of course, by chemically synthesizing the coding sequence, any desired modification can be made simply by substituting the appropriate bases for those encoding the native peptide sequence. The coding sequence can then be provided with appropriate linkers and ligated into expression vectors now commonly available in the art.

25-10-2001

WO99/40934

PCT/US99/03055

-36-

and the regulating vectors used to transform suitable hosts to produce the desired protein.

A number of such vectors and suitable host systems are now available. For example promoter sequences compatible with bacterial hosts are provided in plasmids containing convenient restriction sites for insertion of the desired coding sequence. Typical of such vector plasmids are, for example, pUC8, and pUC13 available from J. Messing, at the University of Minnesota (see, e.g., Messing et al., *Nucleic Acids Res.* 9:309 (1981)) or pBR322, available from New England Biolabs. Suitable promoters include, for example, the beta-lactamase (penicillinase) and lactose (lac) promoter systems (Chang. et al., *Nature* 198:1056 (1977) and the tryptophan (trp) promoter system (Goeddel et al., *Nucleic Acids Res.* 8:4057 (1980)). The resulting expression vectors are transformed into suitable bacterial hosts using the calcium chloride method described by Cohen, et al., *Proc. Natl. Acad. Sci. U.S.A.* 69:2110 (1972). Successful transformants may produce the desired polypeptide fragments at higher levels than those found in strains normally producing the intact pili. Of course, yeast or mammalian cell hosts can also be used, employing suitable vectors and control sequences.

WO 99/40934

PCT/US99/03055

-37-

Table 2
Polypeptide haptens

Organism	Antigen	T or B cell epitope	Sequence	SEQ ID NO
Streptococcus pneumoniae	PspA	B	KLEELSDKIDELDAE	25
Streptococcus pneumoniae	PspA	B	SQKKYDEDQKKTEEKAALEKA ASEEMDKAVAAVQQA	26
Cryptosporidium parvum	P23	B	QDKPADAPAAEAPAAEPAAQQ DKPADA	27
HIV	P24	T	GPKEPFRDYVDRFYKC	28
HIV	GP120	B	RKRIHIGPGRAYITKN	29
Foot and Mouth Disease Virus	VP1	B	YNGECRYNRNAVPLRGDLQV LAQKVARTLP	30
Corynebacterium diphtheriae	toxin	T	FQVVHNSYNRPAYSPGC	31
Borrelia burgdorferi	OspA	T	VEIKEGTVTLKREIDKNGKVT VSIC	32
Borrelia burgdorferi	OspA	T	TLSKNISKSGEVSVELNDC	33
Influenza A8/PR8	HA	T	SSVSSPERFEC	34
Influenza A8/PR8	HA	B	YRNLLWLTEK	35
Yersinia pestis	V Ag	B	DILKVIVDSDMNHHGDARSKLR EELAELTAELKIYSVIQAEIN KHLSSSGTINIHDKSINLMDK NLYGYTDEEIPKASABYKILE KMPQTIIQVDGSEKKIVSIKD FLGSENKRTGALGNILKNSYSY NKDNNELSHFATTCSD	36
Haemophilus influenzae	PBOMP	B	CSSSNNDAAAGNGAAQFGGY	37
Haemophilus influenzae	PBOMP	B	NKLGTVSYGEE	38
Haemophilus influenzae	PBOMP	B	NDEAAYSKNRRAVLAY	39
Moraxella catarrhalis	copB	B	LDIEKDKKKRTDEQLQAEIIDD KYAGKGY	40
Moraxella catarrhalis	copB	B	LDIEKNKKRTEAEIQLQAEIIDD KYAGKGY	41
Moraxella catarrhalis	copB	B	IDIEKKKGKIRTEAELLAELNK DYPGQGY	42

WO 99/40934

PCT/US99/03055

-38-

<i>Porphyromonas gingivalis</i>	HA	B	GVSPKVKDVTVEGSNEFAPV QNLT	43
<i>Porphyromonas gingivalis</i>	HA	B	RIQSTWRQKTVSDLPAKGKYV	44
<i>Trypanosoma cruzi</i>		T	SHNFTLVASVIIEAPSGNTC	45
<i>Trypanosoma cruzi</i>		B	KAAIAPAKAAAAPAKAATAPA	46
<i>Plasmodium falciparum</i>	MSP1	T	SVQIPKVPYPNGIVYC	47
<i>Plasmodium falciparum</i>	MSP1	T	DFNHYYTLKTGLEADC	48
<i>Streptococcus sobrinus</i>	AgI/II	B & T	KPRPIYEAKLAQNQKC	49
<i>Streptococcus sobrinus</i>	AgI/II	B & T	AKADYEAKLAQYEKDLC	50
<i>lymphocytic Choriomeningitis virus</i>	NP	T	RPQASGVYMGNLTAQC	51
<i>Shigella flexneri</i>	Invasin	B	KDRTLIEQK	52
<i>respiratory syncytial virus</i>	G	B	CSICSNNPCTCWAICK	53
<i>Plasmodium vivax</i>	CS	B	GDRADGQPAGDRADGQPAG	54
<i>Clostridium tetani</i>	tox	T	QYIKANSKFIGITELC	55
<i>Entamoeba histolytica</i>	lectin	B	VECASTVCQNDNSCPIIADVE KCNQ	56
<i>Schistosoma japonicum</i>	para	B	DLQSEISLSENGELIRRAKS AESLASELQRDVD	57
<i>Schistosoma mansoni</i>	para	B	DLQSEISLSELENSELIRRAKA AESLASDLQRDVD	58
<i>Plasmodium vivax</i>		B	DRAAGQPAGDRADGQPAG	83
<i>Influenza virus</i>	Inf1	B	CNNPHRIL	84
<i>Influenza virus</i>	Inf1	T	CPKYVKQNTLKLATGMRVPE KQTR	85
<i>Influenza virus</i>	Inf1	B	SIMRSDAPIGTCSSECITPNG SIPNDKPFQNVNKITY	14
<i>Influenza virus</i>	Inf1	B	RGIFGAIAGFIENGWEGMIDG WYGFRHQN	15
<i>Influenza virus</i>	Inf1	B	EKQTRGIFGA	16
<i>Mycobacterium tuberculosis</i>		T	AVLEDPYILLVSSKV	86
<i>Mycobacterium tuberculosis</i>		T	LLVSSKVSTVKDLLP	87

Mycobacterium tuberculosis		T	LLPLLEKVIAGKPL	88
Mycobacterium tuberculosis		T	AILTGGQVISEEVGL	89
Mycobacterium tuberculosis		T	IAFNSGLEPGVVAEK	90
Mycobacterium tuberculosis		T	ARRGLERGLNALADAVKV	91
Mycobacterium tuberculosis		T	EKIGAELVKEVAKK	92
Mycobacterium tuberculosis		T	GLKRGIEKAVEKVTEL	93
Mycobacterium tuberculosis		T	IEDAVRNAKAAVREG	94
Feline leukemia virus	FeLV	B	CDIIGNTWNPSDQEPPGYG	95
Feline leukemia virus	FeLV	B	CIGTVPKTHQALCNETQQGHT	96
Feline leukemia virus	FeLV	B	GNYSNQTNPSSC	97
Feline leukemia virus	FeLV	B	TDIQALEESISALEKSLTSLS E	98
Feline leukemia virus	FeLV		AKLRERLKQRQQLF	99
Feline leukemia virus	FeLV		DSQQGWFEQWFNKPWFTTLI SS	100
Feline leukemia virus	FeLV		QVMTITPPQAMGPNLVLP	101
Feline leukemia virus	FeLV		DQKPPSRQSQIESRVTP	102
Feline leukemia virus	FeLV		RRGLDILFLQEGGLC	103
Feline leukemia virus	FeLV		QEGGLCAALEECQIGGLCAAL KEEC	104
Plasmodium falciparum		B	NANPNANPNANP	105
Circumsporozoite			QAQGDGANAGQP	113

Chemical Hapten. Related chemistry is used

to couple chemical compounds to carrier proteins.

5 Typically, an appropriate functional group for coupling is designed into the chemical compound.

Antibodies to the compound 6-O-phosphocholine hydroxyhexanoate protect against *Streptococcus pneumoniae*. Randy T. Fischer et al. J.

10 Immunol., 154:3373-3382 (1995).

WO 99/40934

PCT/US99/03055

-40-

Table 3
Chemical Haptens

Chemical Hapten	Citation
piperidine N-oxide	U.S. Patent No. 5,304,252
phospholactone or lactamide	U.S. Patent No. 5,248,611
metal ion complexes	U.S. Patent No. 5,236,825
[2.2.1] or [7.2.2] bicyclic ring compounds	U.S. Patent No. 5,208,152
ionically charged hydroxyl-containing compounds	U.S. Patent No. 5,187,086
phosphonate analogs of carboxylate esters	U.S. Patent No. 5,126,258
cocaine analogs	Carrera et al., <i>Nature</i> 378:725 (1995)

5 Carbohydrate Hapten. There are many methods known in the art to couple carrier proteins to polysaccharides. Aldehyde groups can be generated on either the reducing end [Anderson, *Infect. Immun.*, 39:233-238 (1983); Jennings, et al., *J. Immunol.*, 10 127:1011-1018 (1981); Poren et al., *Mol. Immunol.*, 22:907-919 (1985)] or the terminal end [Anderson et al., *J. Immunol.*, 137:1181-1186 (1986); Beuvery et al., *Dev. Bio. Scand.*, 65:197-204 (1986)] of an oligosaccharide or relatively small polysaccharide,

WO 99/40934

PCT/US99/03055

-41-

which can be linked to the carrier protein via reductive amination.

Large polysaccharides can be conjugated by either terminal activation [Anderson et al., J. Immunol., 137:1181-1186 (1986)] or by random activation of several functional groups along the polysaccharide chain [Chu et al., Infect. Immun., 40:245-256 (1983); Gordon, U.S. Patent 4,619,828 (1986); Marburg, U.S. Patent 4,882,317 (1989)].

Random activation of several functional groups along the polysaccharide chain can lead to a conjugate that is highly cross-linked due to random linkages along the polysaccharide chain. The optimal ratio of polysaccharide to carrier protein depend on the particular polysaccharide, the carrier protein, and the conjugate used.

See Dick et al., in Contributions to Microbiology and Immunology, Vol. 10, Cruse et al., eds., (S. Karger: 1989), pp. 48-114; Jennings et al., in Neoglycoconjugates: Preparation and Applications, Lee et al., eds., (Academic Press: 1994), pp. 325-371; Aplin et al., CRC Crit. Rev. Biochem., 10:259-306 (1981); and Stowell et al., Adv. Carbohydr. Chem. Biochem., 37:225-281 (1980) for detailed reviews of methods of conjugation of saccharide to carrier proteins.

The carbohydrate itself can be synthesized by methods known in the art, for example by enzymatic glycoprotein synthesis as described by Witte et al., J. Am. Chem. Soc., 119:2114-2118 (1997).

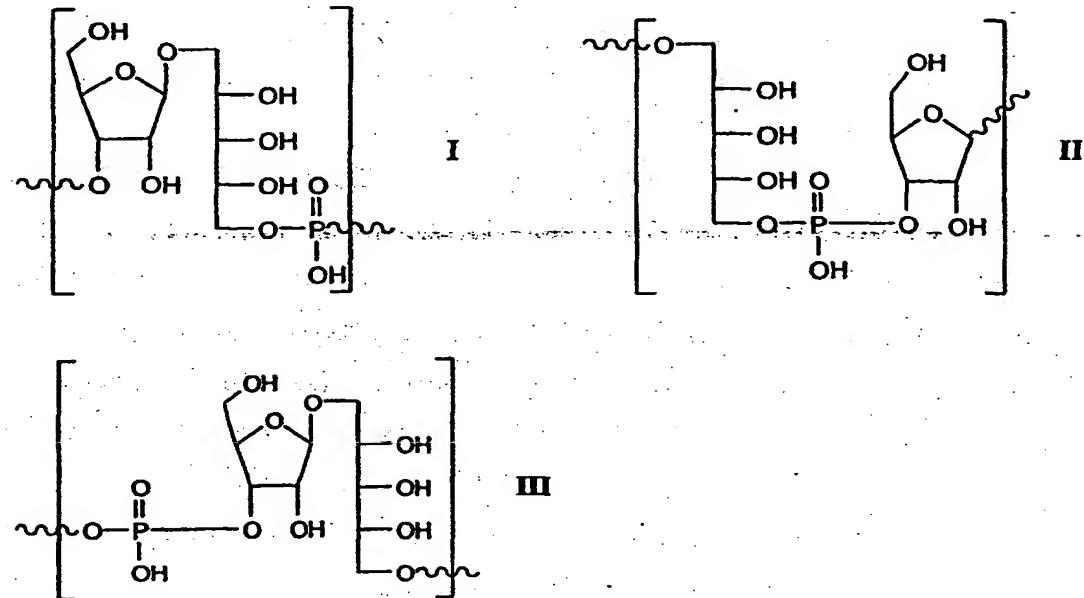
WO.99/40934

PCT/US99/03055

-42-

Several oligosaccharides, synthetic and semi-synthetic, and natural, are discussed in the following paragraphs as examples of oligosaccharides that are contemplated haptens to be used in making a strategically modified core protein conjugate of the present invention.

An oligosaccharide hapten suitable for preparing vaccines for the treatment of *Haemophilus Influenza type b* (Hib) is made up of from 2 to 20 repeats of D-ribose-D-ribitol-phosphate (I, below), D-ribitol-phosphate-D-ribose (II, below), or phosphate-D-ribose-D-ribitol (III, below). Eduard C. Beuvery et al., EP-0 276 516-B1.



U.S. Patent 4,220,717 also discloses a polyribosyl ribitol phosphate (PRP) hapten for *Haemophilus influenzae* type b.

Ellena M. Peterson et al., *Infection and Immunity*, 66(8):3848-3855 (1998), disclose a trisaccharide hapten, α Kdo₍₂₋₈₎ α Kdo₍₂₋₄₎ α Kdo, that

WO 99/40934

PCT/US99/03055

-43-

provides protection from *Chlamydia pneumoniae*.

Chlamydia pneumoniae is a cause of human respiratory infections ranging from pharyngitis to fatal pneumonia. Kdo is 3-deoxy-D-manno-oct-2-uloseonic acid.

Bengt Andersson et al., EP-0 126 043-A1, disclose saccharides that can be used in the treatment, prophylaxis or diagnosis of bacterial infections caused by *Streptococci pneumoniae*. One class of useful saccharides are derived from the disaccharide GlcNAc β 1 3Gal. Andersson et al. also found neolactotetraosylceramide to be useful, which is Gal β 1 4GlcNAc β 1 3Gal β 1 4Glc-Cer.

European Patent No. 0 157 899-B1, the disclosures of which are incorporated herein by reference, discloses the isolation of pneumococcal polysaccharides that are useful in the present invention. The following table lists the pneumococcal culture types that produce capsular polysaccharides useful as haptens in the present invention.

Table 4
Polysaccharide Hapten Sources

Danish Type Nomenclature	U.S. Nomenclature	1978 ATCC Catalogue Number
1	1	6301
2	2	6302
3	3	6303
4	4	6304
5	5	

WO 99/40934

PCT/US99/03055

-44-

6A	6	6306
6B	26	6326
7F	51	10351
8	8	6308
9N	9	6309
9V	68	
10A	34	
11A	43	
12F	12	6312
14	14	6314
15B	54	
17F	17	
18C	56	10356
19A	57	
19F	19	6319
20	20	6320
22F	22	
23F	23	6323
25	25	6325
33F	70	

Moraxella (Branhamella) catarrhalis is a known cause of otitis media and sinusitis in children and lower respiratory tract infections in adults.

5 The lipid A portion of the lipoooligosaccharide surface antigen (LOS) of the bacterium is cleaved at the 3-deoxy-D-manno-octulosonic acid-glucosamine linkage. The cleavage product is treated with mild-alkali to remove ester-linked fatty acids while preserving amide-linked fatty acids to yield

10

WO 99/40934

PCT/US99/03055

-45-

detoxified lipopolysaccharide (dLOS) from *M. catarrhalis*. The dLOS is not immunogenic until it is attached to a protein carrier. Xin-Xing Gu, et al. *Infection and Immunity* 66(5):1891-1897 (1998).

5 Group B streptococci (GBS) is a cause of sepsis, meningitis, and related neurologic disorders in humans. The Capsular polysaccharide-specific antibodies are known to protect human infants from infection. Jennings et al., U.S. 5,795,580. The
10 repeating unit of the GBS capsular polysaccharide type II is: 4)- β -D-GlcNAc-(1 3)-[β -D-Galp(1 6)]- β -D-Galp(1 4)- β -D-GlcP-(1 3)- β -D-GlcP-(1 2)-[α -D-NeupNAc(2 3)]- β -D-Galp-(1 , where the bracketed portion is a branch connected to the immediately
15 following unbracketed subunit. The repeating unit of GBS capsular polysaccharide type V is: 4)-[α -D-NeupNAc-(2 3)- β -D-Galp-(1 4)- β -D-GlcNAc-(1 6)]- α -D-GlcP-(1 4)-[β -D-GlcP-(1 3)]- β -D-Galp-(1 4)- β -D-GlcP-(1 .

20 European patent application No. EU-0 641 568-A1, Dr. Helmut Brade, discloses the method of obtaining ladder-like banding pattern antigen from *Chlamydia trachomatis*, *pneumoniae* and *psittaci*.

25 D. Pathogen-Related Conjugate to the Modified HBc

In one embodiment of the invention, the hapten that is conjugated to strategically modified HBc protein is a B cell determinant of a pathogen. B cell determinants of numerous pathogens are known in
30 the art, and several were illustrated in the

WO 99/40934

PCT/US99/03055

-46-

preceding discussions of polypeptide and carbohydrate haptens.

In preferred embodiments, the hapten is a pathogen-related hapten. The use of a portion of a pathogen's protein sequence or carbohydrate sequence as a hapten has distinct advantages over the exposure to an actual pathogen, and even over a passivated or "killed" version of the pathogen.

Exemplary pathogen-related haptens of particular importance are derived from bacteria such as *B. pertussis*, *S. typosa*, *S. paratyphoid A and B*, *C. diphtheriae*, *C. tetani*, *C. botulinum*, *C. perfringens*, *B. anthracis*, *P. pestis*, *P. multocida*, *V. cholerae*, *N. meningitidis*, *N. gonorrhoea*, *H. influenzae*, *T. palladium*, and the like.

Other exemplary sources of pathogen-related haptens of particular importance are viruses such as poliovirus, adenovirus, parainfluenza virus, measles, mumps, respiratory syncytial virus, influenza virus, equine encephalomyelitis virus, hog cholera virus, Newcastle virus, fowl pox virus, rabies virus, feline and canine distemper viruses, foot and mouth disease virus (FMDV), human and simian immunodeficiency viruses, and the like. Other important sources of pathogen-related haptens include rickettsiae, epidemic and endemic typhus, the spotted fever groups, and the like.

Pathogen-related polypeptide haptens are well-known in art and are discussed in numerous disclosures such as U.S. Pat. Nos. 3,149,036, 3,983,228, and 4,069,313; in Essential Immunology, 3rd

WO 99/40934

PCT/US99/03055

-47-

Ed., by Roit, published by Blackwell Scientific Publications; in Fundamentals of Clinical Immunology, by Alexander and Good, published by W.B. Saunders; and in Immunology, by Bellanti, published by W.B.

5 Saunders.

Particularly preferred pathogen-related haptens are those described in U.S. Pat. Nos. 4,625,015, 4,544,500, 4,545,931, 4,663,436, 4,631,191, 4,629,783 and in Patent Cooperation Treaty 10 International Publication No. WO87/02775 and No. WO87/02892, all of whose disclosures are incorporated herein by reference.

Antibodies that immunoreact with the hepatitis B virus can be obtained by using modified 15 HBC conjugated with a polypeptide hapten that corresponds to the sequence of a determinant portion of HBsAg; in particular, residues 110-137 of the "S" (surface) region disclosed in Gerin et al., Proc. Natl. Acad. Sci. USA, 80:2365 (1983).

20 Another conjugate corresponds to amino acids 93-103 of capsid protein VPI of poliovirus type 1 (PV1, Mahoney strain), analogous to the work by Delpeyroux et al., Science, 233:472-475 (1986). Such a modified HBC conjugate provides antibodies that 25 immunoreact with polio. Other potential haptens from poliovirus type 1, Mahoney and Sabin strains are described in European Patent No. 385610.

In preferred embodiments, the hapten is a 30 pathogen-related hapten that immunoreacts with; i.e., is immunologically bound by, antibodies induced by the pathogen. More preferably, the pathogen-related

-48-

hapten induces an antibody response that provides protection against infection by the pathogen.

Methods for determining the presence of antibodies to an immunogen in a body sample from an 5 immunized animal are well known in the art. Methods for determining the presence of both cross-reactive and protective antibodies are well known in the art.

In another embodiment of the invention, the immune response to the B cell determinant is boosted 10 by also providing a T cell determinant.

For example, U.S. Patent No. 4,882,145 describes T cell stimulating polypeptides derived from the HBV nucleocapsid protein. Other T cell determinants are known in the art and can be used as 15 an operatively linked determinant in a contemplated modified HBC protein or particle.

In a particularly preferred embodiment of the invention, such a T cell determinant is derived from the same pathogen as the B cell determinant that 20 is conjugated to the modified HBC. The T cell determinants of various pathogens are reported in the art.

Although it is preferred that the B and T cell determinants are derived from the same pathogen, 25 it is not necessary that they be from the same protein of that pathogen. For example, the B cell determinant from the VP1 protein of the foot and mouth disease virus (FMDV) can be conjugated to a modified HBC particle, wherein the HBC protein is 30 further modified by having a T cell determinant derived from the VP4 protein of FMDV.

WO 99/40934

PCT/US99/03055

-49-

The additional T cell determinant can be introduced into a modified HBc protein on the genetic level using well-known methods within or at a terminus of the HBc protein sequence (amino or carboxy termini), including as part of the DNA inserted to introduce the chemically-reactive amino acid residue, but preferably as a fusion protein at the carboxy terminus. Alternatively, the additional T cell determinant can be operatively linked to the conjugated B cell determinant or the strategically modified HBc protein.

Exemplary disclosures that describe techniques for genetically engineering a DNA sequence, that can be used to produce a fusion protein of the present invention can be found in: U.S. Pat. Nos. 4,428,941 to Galibert et al., 4,237,224 to Cohen et al.; 4,273,875 to Manis; 4,431,739 to Riggs; 4,363,877 to Goodman et al., and Rodriguez & Tait, *Recombinant DNA Techniques: An Introduction*, The Benjamin-Cummings Publishing Co., Inc. Menlo Park, Calif. (1983), whose disclosures are incorporated by reference.

E. Inocula and Vaccines

In yet another embodiment of the invention, a modified HBc protein or particle conjugated to a hapten (a HBc conjugate) is used as the immunogen of an inoculum that induces production of antibodies that immunoreact with the hapten or as a vaccine to provide protection against the pathogen from which the hapten is derived.

WO 99/40934

PCT/US99/03055

-50-

A contemplated inoculum or vaccine comprises a HBcAg conjugate that is dissolved or dispersed in a pharmaceutically acceptable diluent composition that typically also contains water. When administered in an immunogenic effective amount to an animal such as a mammal (e.g., a mouse, dog, goat, sheep, horse, bovine, monkey, ape, or human) or bird (e.g., a chicken, turkey, duck or goose), an inoculum induces antibodies that immunoreact with the conjugated (pendently-linked) hapten. A vaccine is a type of inoculum in which the hapten is pathogen related and the induced antibodies not only immunoreact with the hapten, but also immunoreact with the pathogen or diseased cell, and neutralize the pathogen or diseased cell with which they immunoreact.

The preparation of inocula and vaccines that contain proteinaceous materials as active ingredients is also well understood in the art. Typically, such inocula or vaccines are prepared as parenterals, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified. The immunogenic active ingredient is often mixed with excipients that are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, an inoculum or vaccine can contain minor amounts of auxiliary

WO 99/40934

PCT/US99/03055

-51-

substances such as wetting or emulsifying agents, pH buffering agents or adjuvants which enhance the immunogenic effectiveness of the composition.

Exemplary adjuvants include complete
5 Freund's adjuvant (CFA) that is not used in humans, incomplete Freund's adjuvant (IFA) and alum, which are materials well known in the art, and are available commercially from several sources. The use of small molecule adjuvants is also contemplated.
10 herein.

Exemplary of one group of small molecule adjuvants are the so-called muramyl dipeptide analogues described in U.S. Patent No. 4,767,842. Another type of small molecule adjuvant described in
15 U.S. Patent No. 4,787,482 that is also useful herein is a 4:1 by volume mixture of squalene or squalane and Aracel™ A (mannide monooleate).

Yet another type of small molecule adjuvant useful herein is a 7-substituted-8-oxo or 8-sulfo-
20 guanosine derivative described in U.S. Patents No. 4,539,205, No. 4,643,992, No. 5,011,828 and No. 5,093,318, whose disclosures are incorporated by reference. Of these materials, 7-allyl-8-oxoguanosine (loxoribine) is particularly preferred.
25 That molecule has been shown to be particularly effective in inducing an antigen-(immunogen-)specific response

Inocula and vaccines are conventionally administered parenterally, by injection, for example,
30 either subcutaneously or intramuscularly. Additional formulations that are suitable for other modes of

-52-

administration include suppositories and, in some cases, oral formulation or by nasal spray. For suppositories, traditional binders and carriers can include, for example, polyalkalene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like.

An inoculum or vaccine composition takes the form of a solution, suspension, tablet, pill, capsule, sustained release formulation or powder, and contains an immunogenic amount of strategically modified HBC protein conjugate or strategically modified HBC protein particle conjugate as active ingredient. In a typical composition, an immunogenic amount of strategically modified HBC protein conjugate or strategically modified HBC protein particle conjugate is about 50 µg to about 2 mg of active ingredient per dose, and more preferably about 100 µg to about 1 mg per dose.

The particles and protein conjugates can be formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable salts, include the acid addition salts (formed with the free amino groups of the peptide) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic,

WO 99/40934

PCT/US99/03055

-53-

oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The inocula or vaccines are administered in a manner compatible with the dosage formulation, and in such amount as are therapeutically effective and immunogenic. The quantity to be administered depends on the subject to be treated, capacity of the subject's immune system to synthesize antibodies, and degree of protection desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. However, suitable dosage ranges are of the order of several hundred micrograms active ingredient per individual. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed in intervals (weeks or months) by a subsequent injection or other administration.

Another embodiment of the invention is a process for inducing antibodies in an animal host comprising the steps of inoculating said animal host with an inoculum. The inoculum used in the process comprises an immunogenic amount of a strategically modified hepatitis B core protein conjugate dissolved or dispersed in a pharmaceutically acceptable

WO 99/40934

PCT/US99/03055

-54-

diluent. The strategically modified hepatitis B core protein conjugate used in the process comprises a hapten pendently linked to a strategically modified hepatitis B core protein. Preferably the 5 strategically modified hepatitis B core protein is in particle form. The strategically modified hepatitis B core protein comprises an amino acid sequence corresponding to the hepatitis B core protein amino acid sequence of SEQ ID NO:2 including the amino acid 10 residues numbered about 10 to about 140 and additionally having an insert in the region corresponding to amino acid residues numbered about 50 to about 100, said insert (i) being 1 to about 40 amino acid residues in length, and (ii) containing a 15 chemically-reactive amino acid residue. The hapten is pendently linked to the strategically modified hepatitis B core protein through said chemically-reactive amino acid residue. Preferably, the hapten is pathogen-related. The animal is maintained for a 20 time sufficient for antibodies to be induced.

The invention is illustrated by the following non-limiting examples.

25 Example 1: Construction of a Modified Hepatitis B Core Protein Expression Vector

Using site-directed mutagenesis, a lysine codon (TTT) was introduced between amino acids D78 and P79 of the HBC gene, along with EcoRI (GAATTC) 30 and SacI (GAGCTC) restriction endonuclease sites to facilitate the genetic insertion of other condons for

WO 99/40934

PCT/US99/03055

-55-

producing strategically modified hybrid HBC particles. The insert thus had an amino acid residue sequence GIQKEL, where the GIQ is an artifact of the EcoRI site and the EL is an artifact of the SacI site. The strategically modified hepatitis B core protein was therefore 155 amino acid residues long. The construction of the pKK322-HBc155-K81 expression plasmid is described below.

Oligonucleotide primers P1F (SEQ ID NO:17) and P1R (SEQ ID NO:18, on the complementary strand) were used to amplify the 5' end of the HBC gene (bases 1-234, amino acids 1-78), and simultaneously incorporate an NcoI restriction site (CCATGG) at the 5' end, and an EcoRI restriction site (GAATTC) at the 3' end of the amplified product. Oligonucleotide primers (SEQ ID NO:19) P2F and P2R (SEQ ID NO:20, on the complementary strand) were used to amplify the 3' end of the HBC gene (bases 232-450, amino acids 79-149), and simultaneously incorporate an EcoRI restriction site (GAATTC) at the 5' end, a SacI restriction site (GAGCTC) adjacent to it, an inserted lysine codon (AAA) between them, and a HindIII restriction site at (AAGCTT) the 3' end of the amplified product.

The two PCR products (encoding amino acids 1-78 and amino acids 79-149) were cleaved with EcoRI, ligated together at their common EcoRI overhangs, cleaved with NcoI and HindIII and cloned into the expression plasmid pKK322 (Pharmacia), using standard techniques. The resulting plasmid was called pKK322-HBc-K81. This plasmid can be used for the expression

WO 99/40934

PCT/US99/03055

-56-

of a strategically modified HBC protein bearing a lysine in the immunodominant loop. The expressed strategically modified HBC protein spontaneously formed particles. The strategically modified HBC of 5 this Example thus had an insert corresponding to position 78 of the HBC of SEQ ID NO:2, a chemically reactive lysine residue at a position corresponding to position 82 of the HBC of SEQ ID NO:2, and was truncated at a position corresponding to position 149 10 of the HBC of SEQ ID NO:2.

WO 99/40934

PCT/US99/03055

-57-

Example 2: Modified Hepatitis B Core Particle Purification

Strategically modified HBc particles of Example 1 were expressed in E. coli typically E. coli BLR or BL21 from Novagen (Madison, Wisconsin) or E. coli TB11 from Amersham (Arlington Heights, Illinois). The transfected E. coli denoted HBc155-K81, were expressed plasmid pKK332-HBc155-K81. The strategically modified HBc particles were purified via Sepharose CL-4B chromatography using established procedures. Because particles purify in a predictable manner, the monitoring of particle elution using simple spectroscopy (OD_{280}), in concert with SDS-PAGE analysis to assess purity of individual fractions prior to pooling, was sufficient to enable the routine purification of electrophoretically pure particles in high yield (5-120 mg/L cell culture). The spherical structure of the pure strategically modified hepatitis B core particles was clearly visible in an electron micrograph.

Example 3: Chemical Coupling of Synthetic Peptides and Modified Hepatitis B Core Particles

The strategically modified hepatitis B core particle product of the expression plasmid pKK332-HBc155-K81 from Example 1 was assayed for its chemical reactivity compared with similarly expressed and purified "wild type" truncated hepatitis B core particle HBc149, which is identical to HBc155-K81

WO 99/40934

PCT/US99/03055

-58-

except that it lacks the introduced lysine residue and flanking five amino acids.

Synthetic peptides (haptens) were chemically conjugated to HBC particles using 5 succinimidyl 4-(N-maleimido-methyl) cyclohexane 1-carboxylate (SMCC), a water-soluble heterobifunctional cross-linking reagent. SMCC is reactive towards both sulfhydryl and primary amino groups, enabling the sequential conjugation of 10 synthetic peptides to HBC particles whose primary amino groups have previously been modified with SMCC. Further, the 11.6 angstrom spacer arm afforded by SMCC helps to reduce steric hindrance between the 15 haptens and the HBC carrier, thereby enabling higher coupling efficiencies.

Briefly, HBC155-K81 and HBC149 particles were separately reacted with a 3-fold excess of SMCC over total amino groups (native amino groups or native amino groups plus the one from the lysine residue of the insert) for 2 hours at room 20 temperature in 50 mM sodium phosphate, pH 7.5, to form maleimide-activated HBC particles. Unreacted SMCC was removed by repeated dialysis against 50 mM sodium phosphate, pH 7.0. The SMCC derivitization of 25 the HBC particles resulted in a minimal molecular weight increase which was not detectable by SDS-PAGE. However, the PAGE analysis did confirm the integrity of the HBC proteins prior to proceeding to the peptide conjugation step.

30 Synthetic peptides to be coupled to the HBC particles were designed such that they had N-terminal



PCT/US99/03055

WO 99/40934

PCT/US99/03055

-59-

cysteine residues to enable directional conjugation of peptide haptens to the primary amine on the side chain of the introduced lysine residue via the cysteine sulfhydryl of the hapten.

5 Table 2 shows the synthetic peptides derived from human cytochrome P450 enzymes that were chemically conjugated to HBC particles. The synthetic peptides were dissolved in 50 mM sodium phosphate, pH 7.0, to a concentration of 10 mg/ml.

10 The synthetic peptides were then added, dropwise, to a 5-fold excess over total amino groups in maleimide-activated strategically modified HBC155-K81 particles, and permitted to react at room temperature, for 2 hours. Maleimide-activated HBC149 particles

15 were reacted with the two 2D6 peptides (206 and 206-C) as controls.

-60-

Table 5
Cytochrome P-450 Haptens

	<u>Peptide Name</u>	<u>Sequence</u>	<u>SEQ ID NO.:</u>
5	1A1 (289-302)	CQEQLDENANVQL	21
	1A2 (291-302)	CSKKGPRASGNLI	22
	2D6 (263-277)	CLTEHRMTWDPAQPPRDLT	23
	3A4 (253-273)	CVKRMKESRLEDTQKHRVDFLQ	24
10	1A1-c	CMQLRS	106
	1A2-c	CRFSIN	107
	2D6-c	CAVPR	108
	2E1-c	CIPRS	109
	2C-c	CFIPV	110
	3A3/4/7-c	CTVSGA	111
15	3A5-c	CTLSGE	112

**Example 4: Analysis of Modified Core
Particles Conjugates**

20 Strategically modified HBC particles pendently linked to cytochrome P-450 determinant haptens of Example 3 were analyzed by SDS-PAGE and immunoblots to determine if synthetic peptides had been successfully conjugated to HBC. The denaturing conditions of the electrophoresis dissemble of particles into their constituent subunits, HBC monomers. Because HBC monomers have a molecular weight of approximately 16,000 Da, it was simple to resolve HBC155-K81 particles chemically conjugated to either 1A1 (289-302), 1A2 (291-302), 2D6 (263-277) or 3A4 (253-273) peptides, as those peptides have a

WO 99/40934

PCT/US99/03055

-61-

relative molecular mass of approximately 2,000 Da and therefore cause a visible increase in the molecular mass of the HBc protein monomers. From the relative intensities of the conjugated and non-conjugated bands on SDS-PAGE, it was revealed that approximately 50 percent of the HBc155-K81 monomers were operatively linked to hapten, whereas only about 5 percent of the "wild type" HBc149 particles were linked to hapten. The marked increase in the observed success in pendently linking hapten to the strategically modified hepatitis B core protein supports the conclusion that the observed linking occurs via the inserted lysine as opposed to a lysine residue that is also present in the "wild type".

The shift in mobility of HBc particles conjugated to shorter C-terminal P450 derived peptides (5 and 6-mers) is not as pronounced in the SDS-PAGE as that of the longer inhibitory peptides, but shifts of approximately 700 Da were clearly evident in successfully coupled HBc155-K81 monomers. The strategically modified HBc 155-K81 protein exhibited markedly enhanced ability to pendently link to a hapten over the "wild type" HBc149 particles, which showed minimal conjugation.

In the model of core particles propounded by Conway et al. of icosahedral particles of either 180 or 240 associated core protein monomers [Nature, 386:91-94 (1997)], dimers of the relatively exposed immunodominant loop regions of the core monomers extend out from the assembled core particle into solution like spikes on a mace. The "spikes" are

-62-

closely arranged spatially on the HBC particles. The strategic location of the introduced lysine residue on the tip of the spike minimizes the propensity for steric constraints to reactions to link haptens to 5 the assembled core particle. A maximum of 50 percent of the strategically modified HBC monomers were successfully conjugated to the synthetic peptides of Cyt P-450. That amount of pendent linkage corresponds to an average of one hapten attached per 10 core particle spike. This proposed distribution of hapten linkage to the strategically modified HBC particle is supported by PAGE results under semi-denaturing conditions that dissemble the particle while maintaining the dimer association.

15 HBC-2D6 particles, prepared by peptide coupling, were examined using immunoblots to confirm the presentation of the 2D6 epitope. When probed with anti-HBC antisera, the chemically coupled particle yielded two different monomers representing 20 particles with and without the 2D6 peptide. Only the upper band of which blotted with anti-2D6 antisera, thereby confirming the correlation between mobility shift and attachment of the 2D6 peptide.

25 Example 6: Strategic Lysine Insertions

To construct HBC particles with inserted lysine residues at every position in the immunodominant, surface-exposed loop region (amino acids 75-85), PCR was used to amplify the 5' and 3' 30 fragments of the HBC gene and a single lysine codon was introduced via the oligonucleotide primers. The

WO 99/40934

PCT/US99/03055

-63-

oligonucleotide primers and the resulting amino acid sequences are shown in SEQ ID NOS: 61-82. The "wild type" sequences are SEQ ID NOS:59-60.

In order to generate lysine inserts at positions 75 to 84 (HBc-K75 through HBc-K84), the pairs of PCR fragments were digested with the restriction endonuclease *MseI*, which recognizes the sequence, AATT. The modified gene was restored by ligating the oligonucleotide primer (containing the lysine) at the convenient *MseI* restriction site located at nucleotides 221-224. For HBc-K85 (SEQ ID NOS:85-86) it was necessary to generate two fragments that were ligated at a common *XhoI* restriction site (CTCGAG) that is not present in the wild type gene, but could be introduced at position 239-244 without altering any amino acids.

Table 6
Lysine insertion mutants of HBc
in the immunodominant loop

Name	Sequence	SEQ ID NO:
Wild Type HBc	TWVGVNLED P ASRDLVVSYV	60
K75	TWVGVN L EDPASRDLVVSYV	62
K76	TWVGVN K LED P ASRDLVVSYV	64
K77	TWVGVN L ED P ASRDLVVSYV	66
K78	TWVGVN L E D PASRDLVVSYV	68
K79	TWVGVN L E D K PASRDLVVSYV	70
K80	TWVGVN L E D P K ASRDLVVSYV	72
K81	TWVGVN L E D P A KSRDLVVSYV	74
K82	TWVGVN L E D P A S KRDLVVSYV	76

WO 99/40934

PCT/US99/03055

-64-

K83	TWGVNLED <u>PASRKDLVVSYV</u>	78
K84	TWGVNLED <u>PASRD<u>K</u>LVVSYV</u>	80
K85	TWGVNLED <u>PASRD<u>L</u>KVVSYV</u>	82

To purify the strategically modified HBC proteins, cleared cell lysates from a 1L fermentation were precipitated with 45% ammonium sulfate and the 5 resultant pellet subjected to gel filtration using Sepharose CL-4B chromatography (2.5cm x 100cm). Particulate HBC has a characteristic elution position when analyzed using this type of column, which was independent of the amino acid insertions made to the 10 particle. The eleven strategically modified HBC particles generated for this study were analyzed using this procedure, and the elution profiles were measured spectrophotometrically at an absorbance of 280 nm.

15 Three of the constructs (HBC-K75, HBC-K77, and HBC-K79) were produced at levels of between 50 and 100 mg/L, which is comparable with typical yields for wild-type, unmodified HBC particles, e.g. HBC149 particles. Four of the constructs (HBC-K76, HBC-K78, 20 HBC-K81, and HBC-K82) were produced at relatively low levels of between 1 and 20 mg/L. Finally, four of the particles (HBC-K80, HBC-K83, HBC-K84, and HBC-K85) were produced at levels deemed to be barely detectable (< 1 mg/L).

WO 99/40934

PCT/US99/03055

-65-

Table 7

Yields of purified lysine-containing
HBC particles from a 1L fermentation

Particle	Yield (mg/L)
HBC-150 (K75)	77
HBC-150 (K76)	5
HBC-150 (K77)	74
HBC-150 (K78)	10
HBC-150 (K79)	94
HBC-150 (K80)	0
HBC-150 (K81)	17
HBC-150 (K82)	1
HBC-150 (K83)	0
HBC-150 (K84)	0
HBC-150 (K85)	0

5

The foregoing description of the invention, including the specific embodiments and examples, is intended to be illustrative of the present invention and is not to be taken as limiting. Numerous other variations and modifications can be effected without departing from the true spirit and scope of the present invention.

WO 99/40934

PCT/US99/03055

-66-

What is claimed:

1. A strategically modified hepatitis B core protein conjugate comprising a strategically modified hepadnavirus nucleocapsid protein pendently linked to a hapten, wherein the strategically modified hepatitis B core protein comprises an amino acid sequence corresponding to the hepatitis B core protein amino acid sequence of SEQ ID NO:2 including the amino acid residues numbered about 10 to about 140 and additionally having an insert in the region corresponding to amino acid residues numbered about 50 to about 100, said insert (i) being 1 to about 40 amino acid residues in length, and (ii) containing a chemically-reactive amino acid residue, said hapten being pendently linked to said chemically-reactive amino acid residue.
2. The stragetically modified hepatitis B core protein conjugate according to claim 1 wherein the insert is in the region corresponding to amino acid residues numbered about 70 to about 90.
3. The stragetically modified hepatitis B core protein conjugate according to claim 1 wherein the insert is in the region corresponding to amino acid residues numbered 78 to 82.
4. The strategically modified hepatitis B core protein conjugate according to claim 1 wherein

2510200

WO 99/40934

PCT/US99/03055

-67-

the hapten is a polypeptide hapten, chemical hapten or carbohydrate hapten.

5. The strategically modified hepatitis B core protein conjugate according to claim 1 wherein the hapten is a pathogen-related hapten.

6. The strategically modified hepatitis B core protein conjugate according to claim 5 wherein
10 the strategically modified hepatitis B core protein further comprises a T cell stimulating amino acid residue sequence operatively linked to the carboxy terminus of said hepatitis B amino acid sequence.

15 7. The strategically modified hepatitis B core protein conjugate according to claim 6 wherein said T cell stimulating amino acid residue sequence is related to the same pathogen as the pendently linked pathogen-related hapten.

20 8. A strategically modified hepatitis B core protein particle comprising a strategically modified hepatitis B core protein subunit having an insert containing a chemically-reactive amino acid side chain, said insert not itself an antigenic determinant.

25 9. A strategically modified hepatitis B core protein particle conjugate comprising a plurality of strategically modified hepatitis B core protein subunits pendently linked to a hapten.

WO 99/40934

PCT/US99/03055

-68-

10. The strategically modified hepatitis B core protein particle conjugate according to claim 9 wherein about 0.1 to about 0.5 of the strategically modified hepatitis B core protein subunits are pendently linked to a hapten.

10 11. The strategically modified hepatitis B core protein particle conjugate according to claim 9 having a plurality of strategically modified hepatitis B core protein subunits that are pendently linked to different haptens.

15 12. The strategically modified hepatitis B core protein particle conjugate according to claim 9 wherein the strategically modified hepatitis B core protein subunit further comprises a T cell stimulating amino acid residue sequence peptide-bonded to the carboxy terminal residue of the amino acid sequence corresponding to said hepatitis B amino acid sequence.

20 25 13. The strategically modified hepatitis B core protein particle conjugate according to claim 12 wherein the T cell stimulating amino acid residue is from the same pathogen as the pendently linked hapten.

30 14. An immunogenic fusion protein conjugate comprising a polypeptide hapten pendently linked to a strategically modified hepatitis B core

-69-

protein that comprises three peptide-linked domains, I, II and III from the N-terminus, wherein

(a) Domain I corresponds to residues about 10 through 50 of the amino acid sequence of hepatitis B core protein of SEQ ID NO:2;

(b) Domain II is bonded to the carboxy terminal residue of Domain I and corresponds to residues 50 to 100 of said amino acid sequence of hepatitis B core protein that further contains a heterologous insert 1 to about 40 amino acid residues in length that includes a chemically-reactive amino acid residue; and

(c) Domain III is bonded to the carboxy terminal residue of Domain II and corresponds to residues 100 to about 140 of the amino acid sequence of hepatitis B core protein.

15. An immunogenic fusion protein conjugate according to claim 14 wherein the strategically modified hepatitis B core protein further comprises a fourth peptide-linked domain, Domain IV, wherein

(d) Domain IV is bonded to the carboxy terminal residue of Domain III and comprises a T cell epitope.

16. A strategically-modified hepatitis B core protein comprising an amino acid sequence corresponding to about amino acid residue 10 to about 30 amino acid residue 140 of the hepatitis B amino acid sequence of SEQ ID NO:2 and having an amino acid

WO 99/40934

PCT/US99/03055

-70-

sequence insert in the region corresponding to about residue 50 to about residue 100 of the hepatitis B, wherein the insert includes a chemically-reactive amino acid residue and the insert itself is not an 5 antigenic determinant.

17. An inoculum comprising an immunogenic amount of a strategically modified hepatitis B core protein conjugate dissolved or dispersed in a pharmaceutically acceptable diluent, said 10 strategically modified hepatitis B core protein conjugate comprising a hapten pendently linked to a strategically modified hepatitis B core protein, wherein the strategically modified hepatitis B core 15 protein comprises an amino acid sequence corresponding to the hepatitis B core protein amino acid sequence of SEQ ID NO:2 including the amino acid residues numbered about 10 to about 140 and additionally having an insert in the region 20 corresponding to amino acid residues numbered about 50 to about 100, said insert (i) being 1 to about 40 amino acid residues in length, and (ii) containing a chemically-reactive amino acid residue, said hapten being pendently linked to said chemically-reactive 25 amino acid residue.

18. The inoculum according to claim 17 wherein said strategically modified hepatitis B core protein conjugate is present in the form of 30 particles.

-71-

19. The inoculum according to claim 18 wherein said hapten is from a pathogen and wherein use of said inoculum in a mammalian host protects said mammalian host from said pathogen.

5

20. A process for inducing antibodies in an animal host comprising the steps of inoculating said animal host with an inoculum that comprises an immunogenic amount of a strategically modified hepatitis B core protein conjugate dissolved or dispersed in a pharmaceutically acceptable diluent, said strategically modified hepatitis B core protein conjugate comprising a hapten pendently linked to a strategically modified hepatitis B core protein, wherein the strategically modified hepatitis B core protein comprises an amino acid sequence corresponding to the hepatitis B core protein amino acid sequence of SEQ ID NO:2 including the amino acid residues numbered about 10 to about 140 and additionally having an insert in the region corresponding to amino acid residues numbered about 50 to about 100, said insert (i) being 1 to about 40 amino acid residues in length, and (ii) containing a chemically-reactive amino acid residue, said hapten being pendently linked to said strategically modified hepatitis B core protein through said chemically-reactive amino acid residue, and maintaining said animal for a time sufficient for antibodies to be induced.

30

WO 99/40934

PCT/US99/03055

-72-

21. The process according to claim 20 wherein said strategically modified hepatitis B core protein conjugate is present in the form of particles.

5

22. process according to claim 21 wherein said hapten is from a pathogen and wherein the antibodies induced in said mammalian host protect said mammalian host from said pathogen.

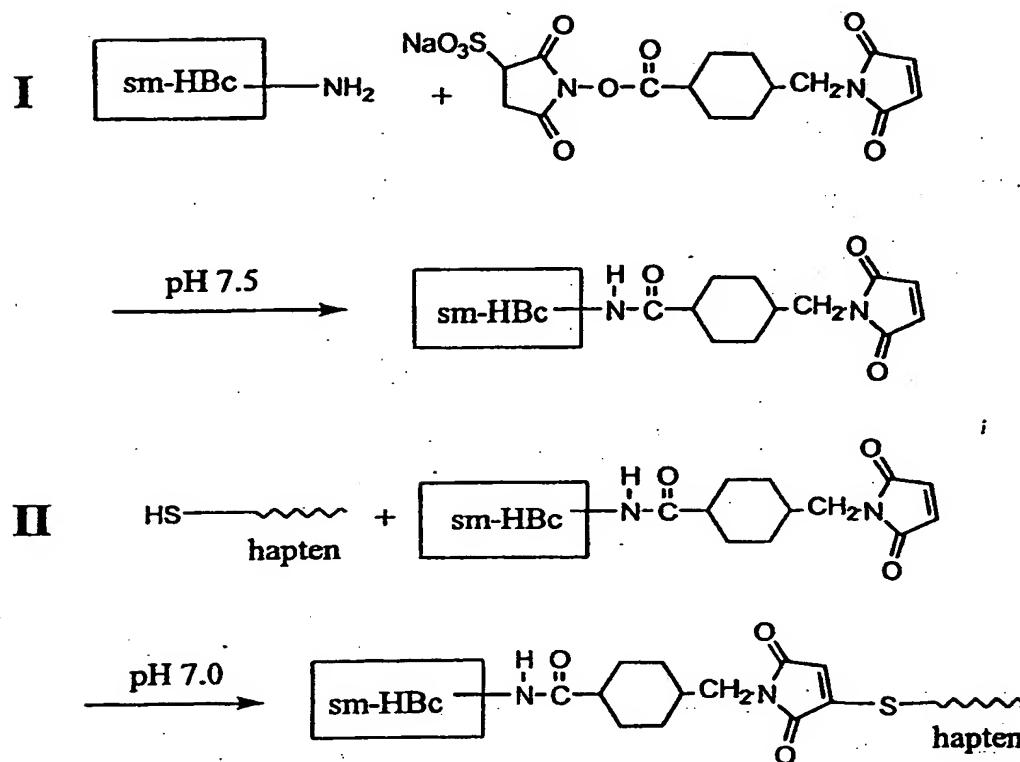
10

WO 99/40934

PCT/US99/03055

1/1

Scheme 1



WO 99/40934

PCT/US99/03055

SEQUENCE LISTING

<110> Birkett, Ashley J.
Immune Complex Inc.

<120> Strategically Modified Hepatitis B Core Proteins and
their Derivatives

<130> SYN-101 4564/69529

<140> PCT/US99/
<141> 1999-02-11

<150> 60/074537
<151> 1998-02-12

<160> 113

<170> PatentIn Ver. 2.0

<210> 1
<211> 553
<212> DNA
<213> Hepatitis B virus

<220>
<221> CDS
<222> (1)..(549)

<300>
<303> Nature
<304> 281
<306> 646-
<307> 1979

<400> 1

atg gac atc gac cct tat aaa gaa ttt gga gct act gtg gag tta ctc 48
Met Asp Ile Asp Pro Tyr Lys Glu Phe Gly Ala Thr Val Glu Leu Leu
1 5 10 15

tgc ttt ttg cct tct gac ttc ttt cct tca gta cga gat ctt cta gat 96
Ser Phe Leu Pro Ser Asp Phe Pro Ser Val Arg Asp Leu Leu Asp
20 25 30

acc gcc tca gct ctg tat cgg gaa gcc tta gag tct cct gag cat tgt 144
Thr Ala Ser Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro Glu His Cys
35 40 45

WO 99/40934

PCT/US99/03055

tca cct cac cat act gca ctc agg caa gca att ctt tgc tgg ggg gaa	192			
Ser Pro His His Thr Ala Leu Arg Gln Ala Ile Leu Cys Trp Gly Glu				
50	55	60		
cta atg act cta gct acc tgg gtg ggt gtt aat ttg gaa gat cca gcg	240			
Leu Met Thr Leu Ala Thr Trp Val Gly Val Asn Leu Glu Asp Pro Ala				
65	70	75	80	
tct aga gac cta gta gtc agt tat gtc aac act aat atg ggc cta aag	288			
Ser Arg Asp Leu Val Val Ser Tyr Val Asn Thr Asn Met Gly Leu Lys				
85	90	95		
tcc agg caa ctc ttg tgg ttt cac att tct tgt ctc act ttt gga aga	336			
Phe Arg Gln Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Arg				
100	105	110		
gaa aca gtt ata gag tat ttg gtg tct ttc gga gtg tgg att cgc act	384			
Glu Thr Val Ile Glu Tyr Leu Val Ser Phe Gly Val Trp Ile Arg Thr				
115	120	125		
cct cca gct tat aga cca cca aat gcc cct atc cta tca aca ctt ccg	432			
Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro				
130	135	140		
gag act act gtt gtt aga cga cga ggc agg tcc cct aga aga aga act	480			
Glu Thr Thr Val Val Arg Arg Arg Gly Arg Ser Pro Arg Arg Arg Thr				
145	150	155	160	
ccc tcg cct cgc aga cga agg tct caa tcg ccg cgt cgc aga aga tct	528			
Pro Ser Pro Arg Arg Arg Ser Gln Ser Pro Arg Arg Arg Arg Ser				
165	170	175		
caa tct cgg gaa tct caa tgt tagt	553			
Gln Ser Arg Glu Ser Gln Cys				
180				
<210> 2				
<211> 183				
<212> PRT				
<213> Hepatitis B virus				
<400> 2				
Met Asp Ile Asp Pro Tyr Lys Glu Phe Gly Ala Thr Val Glu Leu Leu				
1	5	10	15	
Ser Phe Leu Pro Ser Asp Phe Phe Pro Ser Val Arg Asp Leu Leu Asp				
20	25	30		

WO 99/40934

PCT/US99/03055

Thr Ala Ser Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro Glu His Cys
 35 40 45

Ser Pro His His Thr Ala Leu Arg Gln Ala Ile Leu Cys Trp Gly Glu
 50 55 60

Leu Met Thr Leu Ala Thr Trp Val Gly Val Asn Leu Glu Asp Pro Ala
 65 70 75 80

Ser Arg Asp Leu Val Val Ser Tyr Val Asn Thr Asn Met Gly Leu Lys
 85 90 95

Phe Arg Gln Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Arg
 100 105 110

Glu Thr Val Ile Glu Tyr Leu Val Ser Phe Gly Val Trp Ile Arg Thr
 115 120 125

Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro
 130 135 140

Glu Thr Thr Val Val Arg Arg Arg Gly Arg Ser Pro Arg Arg Arg Thr
 145 150 155 160

Pro Ser Pro Arg Arg Arg Ser Gln Ser Pro Arg Arg Arg Ser
 165 170 175

Gln Ser Arg Glu Ser Gln Cys
 180

<210> 3

<211> 552

<212> DNA

<213> Hepatitis B virus

<220>

<221> CDS

<222> (1)..(549)

<300>

<303> Nucleic Acids Res.

<304> 11

<306> 1747-

<307> 1983

<400> 3

atg gac att gac ccg tat aaa gaa ttt gga gca tct gtg gag tta ctc 48

WO 99/40934

PCT/US99/03055

Met Asp Ile Asp Pro Tyr Lys Glu Phe Gly Ala Ser Val Glu L u Leu
 1 5 10 15

tct ttt ttg cct tct gac ttc ttt ccg tct att cga gat ctc ctt gac 96
 Ser Phe Leu Pro Ser Asp Phe Phe Pro Ser Ile Arg Asp Leu Leu Asp
 20 25 30

acc gcc tct gct ctg tat cgg gag gcc tta gag tct ccg gaa cat tgt 144
 Thr Ala Ser Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro Glu His Cys
 35 40 45

tca cct cac cat aca gca ctc agg caa gct att ctg tgt tgg ggt gag 192
 Ser Pro His His Thr Ala Leu Arg Gln Ala Ile Leu Cys Trp Gly Glu
 50 55 60

tta atg aat ctg gcc acc tgg gtg gga agt aat ttg gaa gac cca gca 240
 Leu Met Asn Leu Ala Thr Trp Val Gly Ser Asn Leu Glu Asp Pro Ala
 65 70 75 80

tcc agg gaa tta gta gtc agc tat gtc aat gtt aat atg ggc cta aaa 288
 Ser Arg Glu Leu Val Val Ser Tyr Val Asn Val Asn Met Gly Leu Lys
 85 90 95

atc aga caa cta ttg tgg ttt cac att tcc tgc ctt act ttt gga aga 336
 Ile Arg Gln Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Arg
 100 105 110

gaa act gtt ttg gag tat ttg gta tct ttt gga gtg tgg att cgc act 384
 Glu Thr Val Leu Glu Tyr Leu Val Ser Phe Gly Val Trp Ile Arg Thr
 115 120 125

cct ccc gct tac aga cca cca aat gcc cct atc tta tca aca ctt ccg 432
 Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro
 130 135 140

gaa act act gtt aga cga cga ggc agg tcc cct aga aga aga act 480
 Glu Thr Thr Val Val Arg Arg Arg Gly Arg Ser Pro Arg Arg Arg Thr
 145 150 155 160

ccc tcg cct cgc aga cga agg tct caa tcg ccg cgt cgc aga aga tct 528
 Pro Ser Pro Arg Arg Arg Ser Gln Ser Pro Arg Arg Arg Arg Ser
 165 170 175

caa tct cgg gaa tct caa tgt tag 552
 Gln Ser Arg Glu Ser Gln Cys
 180

WO 99/40934

PCT/US99/03055

<210> 4

<211> 183

<212> PRT

<213> Hepatitis B virus

<400> 4

Met Asp Ile Asp Pro Tyr Lys Glu Phe Gly Ala Ser Val Val Glu Leu Leu
 1 5 10 15

Ser Phe Leu Pro Ser Asp Phe Phe Pro Ser Ile Arg Asp Leu Leu Asp
 20 25 30

Thr Ala Ser Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro Glu His Cys
 35 40 45

Ser Pro His His Thr Ala Leu Arg Gln Ala Ile Leu Cys Trp Gly Glu
 50 55 60

Leu Met Asn Leu Ala Thr Trp Val Gly Ser Asn Leu Glu Asp Pro Ala
 65 70 75 80

Ser Arg Glu Leu Val Val Ser Tyr Val Asn Val Asn Met Gly Leu Lys
 85 90 95

Ile Arg Gln Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Arg
 100 105 110

Glu Thr Val Leu Glu Tyr Leu Val Ser Phe Gly Val Trp Ile Arg Thr
 115 120 125

Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro
 130 135 140

Glu Thr Thr Val Val Arg Arg Arg Gly Arg Ser Pro Arg Arg Arg Thr
 145 150 155 160

Pro Ser Pro Arg Arg Arg Arg Ser Gln Ser Pro Arg Arg Arg Arg Ser
 165 170 175

Gln Ser Arg Glu Ser Gln Cys
 180

<210> 5

<211> 558

<212> DNA

<213> Hepatitis B virus

WO 99/40934

PCT/US99/03055

<220>

<221> CDS

<222> (1)..(555)

<300>

<303> Nucleic Acids Res.

<304> 11

<306> 1747-

<307> 1983

<400> 5

atg gac att gac cct tat aaa gaa ttt gga gct act gtg gag tta ctc 48
 Met Asp Ile Asp Pro Tyr Lys Glu Phe Gly Ala Thr Val Glu Leu Leu
 1 5 10 15

tcg ttt ttg cct tct gac ttc ttt cct tcc gta cga gat ctc cta gac 96
 Ser Phe Leu Pro Ser Asp Phe Phe Pro Ser Val Arg Asp Leu Leu Asp
 20 25 30

acc gcc tca gct ctg tat cga gaa gcc tta gag tct cct gag cat tgc 144
 Thr Ala Ser Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro Glu His Cys
 35 40 45

tca cct cac cat act gca ctc agg caa gcc att ctc tgc tgg ggg gaa 192
 Ser Pro His His Thr Ala Leu Arg Gln Ala Ile Leu Cys Trp Gly Glu
 50 55 60

ttg atg act cta gct acc tgg gtg ggt aat aat ttg caa gat cca gca 240
 Leu Met Thr Leu Ala Thr Trp Val Gly Asn Asn Leu Gln Asp Pro Ala
 65 70 75 80

tcc aga gat cta gta gtc aat tat gtt aat act aac atg ggt tta aag 288
 Ser Arg Asp Leu Val Val Asn Tyr Val Asn Thr Asn Met Gly Leu Lys
 85 90 95

atc agg caa cta ttg tgg ttt cat ata tct tgc ctt act ttt gga aga 336
 Ile Arg Gln Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Arg
 100 105 110

gag act gta ctt gaa tat ttg gtc tct ttc gga gtg tgg att cgc act 384
 Glu Thr Val Leu Glu Tyr Leu Val Ser Phe Gly Val Trp Ile Arg Thr
 115 120 125

cct cca gcc tat aga cca cca aat gcc cct atc tta tca aca ctt ccg 432
 Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro
 130 135 140

gaa act act gtt gtt aga cga cgg gac cga ggc agg tcc cct aga aga 480

WO 99/40934

PCT/US99/03055

Glu Thr Thr Val Val Arg Arg Asp Arg Gly Arg Ser Pro Arg Arg
 145 150 155 160
 aga act ccc tcc cct cgc aga cgc aga tct caa tcg ccg cgt cgc aga 528
 Arg Thr Pro Ser Pro Arg Arg Ser Gln Ser Pro Arg Arg Arg
 165 170 175
 aga tct caa tct cgg gaa tct caa tgt tag 558
 Arg Ser Gln Ser Arg Glu Ser Gln Cys
 180 185

<210> 6
 <211> 185
 <212> PRT
 <213> Hepatitis B virus

<400> 6
 Met Asp Ile Asp Pro Tyr Lys Glu Phe Gly Ala Thr Val Glu Leu Leu
 1 5 10 15
 Ser Phe Leu Pro Ser Asp Phe Phe Pro Ser Val Arg Asp Leu Leu Asp
 20 25 30
 Thr Ala Ser Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro Glu His Cys
 35 40 45
 Ser Pro His His Thr Ala Leu Arg Gln Ala Ile Leu Cys Trp Gly Glu
 50 55 60
 Leu Met Thr Leu Ala Thr Trp Val Gly Asn Asn Leu Gln Asp Pro Ala
 65 70 75 80
 Ser Arg Asp Leu Val Val Asn Tyr Val Asn Thr Asn Met Gly Leu Lys
 85 90 95
 Ile Arg Gln Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Arg
 100 105 110
 Glu Thr Val Leu Glu Tyr Leu Val Ser Phe Gly Val Trp Ile Arg Thr
 115 120 125
 Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro
 130 135 140
 Glu Thr Thr Val Val Arg Arg Asp Arg Gly Arg Ser Pro Arg Arg
 145 150 155 160

WO 99/40934

PCT/US99/03055

Arg Thr Pro Ser Pro Arg Arg Arg Arg Ser Gln Ser Pro Arg Arg Arg
 165 170 175

Arg Ser Gln Ser Arg Glu Ser Gln Cys
 180 185

<210> 7
 <211> 188
 <212> PRT
 <213> Hepatitis B virus

<300>
 <301> Schodel, Florian
 et al.,
 <302> Animal Hepatitis B Viruses
 <303> Adv. Viral Oncol.
 <304> 8
 <306> 73-102
 <307> 1989

<400> 7
 Met Asp Ile Asp Pro Tyr Lys Glu Phe Gly Ser Ser Tyr Gln Leu Leu
 1 5 10 15

Asn Phe Leu Pro Leu Asp Phe Phe Pro Asp Leu Asn Ala Leu Val Asp
 20 25 30

Thr Ala Ala Ala Leu Tyr Glu Glu Glu Leu Thr Gly Arg Glu His Cys
 35 40 45

Ser Pro His His Thr Ala Ile Arg Gln Ala Leu Val Cys Trp Asp Glu
 50 55 60

Leu Thr Lys Leu Ile Ala Trp Met Ser Ser Asn Ile Thr Ser Glu Gln
 65 70 75 80

Val Arg Thr Ile Ile Val Asn His Val Asn Asp Thr Trp Gly Leu Lys
 85 90 95

Val Arg Gln Ser Leu Trp Phe His Leu Ser Cys Leu Thr Phe Gly Gln
 100 105 110

His Thr Val Gln Glu Phe Leu Val Ser Phe Gly Val Trp Ile Arg Thr
 115 120 125

Pro Ala Pro Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro
 130 135 140

Glu His Thr Val Ile Arg Arg Arg Gly Gly Ala Arg Ala Ser Arg Ser
145 150 155 160

Pro Arg Arg Arg Thr Pro Ser Pro Arg Arg Arg Ser Gln Ser Pro
165 170 175

Arg Arg Arg Arg Ser Gln Ser Pro Ser Ala Asn Cys
180 185

<210> 8

<211> 654

<212> DNA

<213> Hepatitis B virus

<220>

<221> CDS

<222> (1)..(651)

<300>

<302> Animal Hepatitis B Viruses

<303> Adv. Viral Oncol.

<304> 8

<306> 73-102

<307> 1989

<400> 8

atg tat ctt ttt cac ctg tgc ctt gtt ttt gcc tgg gtt cca tgg cct 48
Met Tyr Leu Phe His Leu Cys Leu Val Phe Ala Cys Val Pro Cys Pro
1 5 10 15

act gtt caa gcc tcc aag ctg tgc ctt gga tgg ctt tgg gac atg gac 96
Thr Val Gln Ala Ser Lys Leu Cys Leu Gly Trp Leu Trp Asp Met Asp
20 25 30

ata gat ccc tat aaa gaa ttt ggt tct tct tat cag ttg ttg aat ttt 144
Ile Asp Pro Tyr Lys Glu Phe Gly Ser Ser Tyr Gln Leu Leu Asn Phe
35 40 45

ctt cct ttg gac ttt cct gat ctc aat gca ttg gtg gac act gct 192
Leu Pro Leu Asp Phe Phe Pro Asp Leu Asn Ala Leu Val Asp Thr Ala
50 55 60

gct gct ctt tat gaa gaa gaa tta aca ggt agg gag cat tgg tct cct 240
Ala Ala Leu Tyr Glu Glu Glu Leu Thr Gly Arg Glu His Cys Ser Pro
65 70 75 80

WO 99/40934

PCT/US99/03055

cat cat act gct att aga cag gcc tta gtg tgt tgg gaa gaa tta act 288
 His His Thr Ala Ile Arg Gln Ala Leu Val Cys Trp Glu Glu Leu Thr
 85 90 95

aga tta att aca tgg atg agt gaa aat aca aca gaa gaa gtt aga aga 336
 Arg Leu Ile Thr Trp Met Ser Glu Asn Thr Thr Glu Glu Val Arg Arg
 100 105 110

att att gtt gat cat gtc aat aat act tgg gga ctt aaa gta aga cag 384
 Ile Ile Val Asp His Val Asn Asn Thr Trp Gly Leu Lys Val Arg Gln
 115 120 125

act tta tgg ttt cat tta tca tgt ctt act ttt gga caa cac aca gtt 432
 Thr Leu Trp Phe His Leu Ser Cys Leu Thr Phe Gly Gln His Thr Val
 130 135 140

caa gaa ttt ttg gtt agt ttt gga gta tgg att aga act cca gct cct 480
 Gln Glu Phe Leu Val Ser Phe Gly Val Trp Ile Arg Thr Pro Ala Pro
 145 150 155 160

tat aga cca cct aat gca ccc att tta tca act ctt ccg gaa cat aca 528
 Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro Glu His Thr
 165 170 175

gtc att agg aga aga gga ggt tca aga gct gct agg tcc ccc cga aga 576
 Val Ile Arg Arg Gly Ser Arg Ala Ala Arg Ser Pro Arg Arg
 180 185 190

cgc act ccc tct cct cgc agg aga agg tct caa tca ccg cgt cgc aga 624
 Arg Thr Pro Ser Pro Arg Arg Arg Ser Gln Ser Pro Arg Arg Arg
 195 200 205

cgc tct caa tct cca gct tcc aac tgc tga 654
 Arg Ser Gln Ser Pro Ala Ser Asn Cys
 210 215

<210> 9
 <211> 217
 <212> PRT
 <213> Hepatitis B virus

<400> 9
 Met Tyr Leu Phe His Leu Cys Leu Val Phe Ala Cys Val Pro Cys Pro
 1 5 10 15

Thr Val Gln Ala Ser Lys Leu Cys Leu Gly Trp Leu Trp Asp Met Asp
 20 25 30

WO 99/40934

PCT/US99/03055

Ile Asp Pro Tyr Lys Glu Phe Gly Ser Ser Tyr Gln Leu Leu Asn Phe
35 40 45

Leu Pro Leu Asp Phe Phe Pro Asp Leu Asn Ala Leu Val Asp Thr Ala
50 55 60

Ala Ala Leu Tyr Glu Glu Glu Leu Thr Gly Arg Glu His Cys Ser Pro
65 70 75 80

His His Thr Ala Ile Arg Gln Ala Leu Val Cys Trp Glu Glu Leu Thr
85 90 95

Arg Leu Ile Thr Trp Met Ser Glu Asn Thr Thr Glu Glu Val Arg Arg
100 105 110

Ile Ile Val Asp His Val Asn Asn Thr Trp Gly Leu Lys Val Arg Gln
115 120 125

Thr Leu Trp Phe His Leu Ser Cys Leu Thr Phe Gly Gln His Thr Val
130 135 140

Gln Glu Phe Leu Val Ser Phe Gly Val Trp Ile Arg Thr Pro Ala Pro
145 150 155 160

Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro Glu His Thr
165 170 175

Val Ile Arg Arg Arg Gly Gly Ser Arg Ala Ala Arg Ser Pro Arg Arg
180 185 190

Arg Thr Pro Ser Pro Arg Arg Arg Ser Gln Ser Pro Arg Arg Arg
195 200 205

Arg Ser Gln Ser Pro Ala Ser Asn Cys
210 215

<210> 10

<211> 918

<212> DNA

<213> Hepatitis B virus

<220>

<221> CDS

<222> (1)..(915)

<400> 10

WO 99/40934

PCT/US99/03055

atg tgg tct cta aga tta cac ccc ttc cca ttc gga gct gcg tgc caa 48
 Met Trp Ser Leu Arg Leu His Pro Ser Pro Phe Gly Ala Ala Cys Gln
 1 5 10 15

ggt atc ttt acg tcg acc tcg ctg ttg ttc ctt gtg act gta cct ttg 96
 Gly Ile Phe Thr Ser Thr Ser Leu Leu Phe Leu Val Thr Val Pro Leu
 20 25 30

gta tgt acc att gtt tat gat tct tgc tta tat atg gat gtc aat gct 144
 Val Cys Thr Ile Val Tyr Asp Ser Cys Leu Tyr Met Asp Val Asn Ala
 35 40 45

tca aga gct tta gca aat gta tat gat ctg cca gat gat ttc ttt cct 192
 Ser Arg Ala Leu Ala Asn Val Tyr Asp Leu Pro Asp Asp Phe Phe Pro
 50 55 60

cag att gat gat ctt gtt aga gat gct aag gat gct tta gaa cct tat 240
 Gln Ile Asp Asp Leu Val Arg Asp Ala Lys Asp Ala Leu Glu Pro Tyr
 65 70 75 80

tgg aaa gcc gaa aca ata aag aaa cat gtt tta att gct act cac ttt 288
 Trp Lys Ala Glu Thr Ile Lys Lys His Val Leu Ile Ala Thr His Phe
 85 90 95

gtg gat ttg att gag gac ttc tgg cag acc act cag ggt atg agc caa 336
 Val Asp Leu Ile Glu Asp Phe Trp Gln Thr Thr Gln Gly Met Ser Gln
 100 105 110

att gca gac gcc ctc cga gca gta att cca cct act acc gta cca gta 384
 Ile Ala Asp Ala Leu Arg Ala Val Ile Pro Pro Thr Thr Val Pro Val
 115 120 125

ccg gag ggt ttt ctc att act cat agt gag gca gaa gag atc ccc ttg 432
 Pro Glu Gly Phe Leu Ile Thr His Ser Glu Ala Glu Glu Ile Pro Leu
 130 135 140

aac gat ctc ttt tca aatcaa gag gag agg ata gtc aat ttc caa cct 480
 Asn Asp Leu Phe Ser Asn Gln Glu Glu Arg Ile Val Asn Phe Gln Pro
 145 150 155 160

gac tat ccc att aca gct aga att cat acc cac tta cgt gtt tat act 528
 Asp Tyr Pro Ile Thr Ala Arg Ile His Thr His Leu Arg Val Tyr Thr
 165 170 175

aaa ttg aat gaa caa gct ttg gac aaa gct cgc aga ttg ctt tgg tgg 576
 Lys Leu Asn Glu Gln Ala Leu Asp Lys Ala Arg Arg Leu Leu Trp Trp
 180 185 190

WO 99/40934

PCT/US99/03055

cat tac aat tgc ctc ctc tgg gga gaa gcc act gtt aca aat tat att 624
 His Tyr Asn Cys Leu Leu Trp Gly Glu Ala Thr Val Thr Asn Tyr Ile
 195 200 205

tct cgc ctc cgt act tgg ctt tct act ccc gaa aaa tat cga ggc aag 672
 Ser Arg Leu Arg Thr Trp Leu Ser Thr Pro Glu Lys Tyr Arg Gly Lys
 210 215 220

gat gcc cca acc att gaa gca atc act aga cca atc cag gtg gct caa 720
 Asp Ala Pro Thr Ile Glu Ala Ile Thr Arg Pro Ile Gln Val Ala Gln
 225. 230 235 240

gga ggc aga aat caa act aag gga act aga aaa cct cgt gga ctc gaa 768
 Gly Gly Arg Asn Gln Thr Lys Gly Thr Arg Lys Pro Arg Gly Leu Glu
 245 250 255

cct aga aga cga aag gtt aaa acc aca gtt gtc tat ggg aga aga cgt 816
 Pro Arg Arg Arg Lys Val Lys Thr Thr Val Val Tyr Gly Arg Arg Arg
 260 265 270

tct aag tcc cga ggc agg aga tcc tct cca caa cgt gcg ggc tcc 864
 Ser Lys Ser Arg Gly Arg Arg Ser Ser Pro Ser Gln Arg Ala Gly Ser
 275 280 285

cct ctc cca cgt aat cgg gga aac cag aca cga tcc ccc tca cct agg 912
 Pro Leu Pro Arg Asn Arg Gly Asn Gln Thr Arg Ser Pro Ser Pro Arg
 290 295 300

gaa tag 918
 Glu
 305

<210> 11
 <211> 305
 <212> PRT
 <213> Hepatitis B virus

<400> 11
 Met Trp Ser Leu Arg Leu His Pro Ser Pro Phe Gly Ala Ala Cys Gln
 1 5 10 15

Gly Ile Phe Thr Ser Thr Ser Leu Leu Phe Leu Val Thr Val Pro Leu
 20 25 30

Val Cys Thr Ile Val Tyr Asp Ser Cys Leu Tyr M t Asp Val Asn Ala
 35 40 45

WO 99/40934

PCT/US99/03055

Ser Arg Ala Leu Ala Asn Val Tyr Asp Leu Pro Asp Asp Phe Phe Pro
 50 55 60

Gln Ile Asp Asp Leu Val Arg Asp Ala Lys Asp Ala Leu Glu Pro Tyr
 65 70 75 ~ 80

Trp Lys Ala Glu Thr Ile Lys Lys His Val Leu Ile Ala Thr His Phe
 85 90 95

Val Asp Leu Ile Glu Asp Phe Trp Gln Thr Thr Gln Gly Met Ser Gln
 100 105 110

Ile Ala Asp Ala Leu Arg Ala Val Ile Pro Pro Thr Thr Val Pro Val
 115 120 125

Pro Glu Gly Phe Leu Ile Thr His Ser Glu Ala Glu Glu Ile Pro Leu
 130 135 140

Asn Asp Leu Phe Ser Asn Gln Glu Glu Arg Ile Val Asn Phe Gln Pro
 145 150 155 160

Asp Tyr Pro Ile Thr Ala Arg Ile His Thr His Leu Arg Val Tyr Thr
 165 170 175

Lys Leu Asn Glu Gln Ala Leu Asp Lys Ala Arg Arg Leu Leu Trp Trp
 180 185 190

His Tyr Asn Cys Leu Leu Trp Gly Glu Ala Thr Val Thr Asn Tyr Ile
 195 200 205

Ser Arg Leu Arg Thr Trp Leu Ser Thr Pro Glu Lys Tyr Arg Gly Lys
 210 215 220

Asp Ala Pro Thr Ile Glu Ala Ile Thr Arg Pro Ile Gln Val Ala Gln
 225 230 235 240

Gly Gly Arg Asn Gln Thr Lys Gly Thr Arg Lys Pro Arg Gly Leu Glu
 245 250 255

Pro Arg Arg Arg Lys Val Lys Thr Thr Val Val Tyr Gly Arg Arg Arg
 260 265 270

Ser Lys Ser Arg Gly Arg Arg Ser Ser Pro Ser Gln Arg Ala Gly Ser
 275 280 285

Pro Leu Pro Arg Asn Arg Gly Asn Gln Thr Arg Ser Pro Ser Pro Arg
 290 295 300

WO 99/40934

Glu
305

<210> 12
<211> 918
<212> DNA
<213> Hepatitis B virus

<220>
<221> CDS
<222> (1)..(915)

<400> 12
atg tgg aac tta aga att aca ccc ctc tcc ttc gga gct gct tgc caa 48
Met Trp Asn Leu Arg Ile Thr Pro Leu Ser Phe Gly Ala Ala Cys Gln
1 5 10 15

ggt atc ttt acg tct aca ttg ctg ttg tcg tgt gtg act gta cct ttg 96
Gly Ile Phe Thr Ser Thr Leu Leu Leu Ser Cys Val Thr Val Pro Leu
20 25 30

gta tgt acc att gtt tat gat tct tgc tta tat atg gat atc aat gct 144
Val Cys Thr Ile Val Tyr Asp Ser Cys Leu Tyr Met Asp Ile Asn Ala
35 40 45

tct aga gcc tta gcc aat gtg tat gat cta cca gat gat ttc ttt cca 192
Ser Arg Ala Leu Ala Asn Val Tyr Asp Leu Pro Asp Asp Phe Phe Pro
50 55 60

aaa ata gat gat ctt gtt aga gat gct aaa gac gct tta gag cct tat 240
Lys Ile Asp Asp Leu Val Arg Asp Ala Lys Asp Ala Leu Glu Pro Tyr
65 70 75 80

tgg aaa tca gat tca ata aag aaa cat gtt ttg att gca act cac ttt 288
Trp Lys Ser Asp Ser Ile Lys Lys His Val Leu Ile Ala Thr His Phe
85 90 95

gtg gat ctc att gaa gac ttc tgg cag act aca cag ggc atg cat gaa 336
Val Asp Leu Ile Glu Asp Phe Trp Gln Thr Gln Gly Met His Glu
100 105 110

ata gcc gaa tca tta aga gct gtt ata cct ccc act act act cct gtt 384
Ile Ala Glu Ser Leu Arg Ala Val Ile Pro Pro Thr Thr Pro Val
115 120 125

cca ccg ggt tat ctt att cag cac gag gaa gct gaa gag ata cct ttg 432
Pro Pro Gly Tyr Leu Ile Gln His Glu Glu Ala Glu Glu Ile Pro Leu

WO 99/40934

PCT/US99/03055

130

135

140

gga gat tta ttt aaa cac cca gaa gaa agg ata gta agt ttc cca ccc 480

Gly Asp Leu Phe Lys His Gln Glu Glu Arg Ile Val Ser Phe Gln Pro

145

150

155

160

gac tat ccg att acg gct aga att cat gct cat ttg aaa gct tat gca 528

Asp Tyr Pro Ile Thr Ala Arg Ile His Ala His Leu Lys Ala Tyr Ala

165

170

175

aaa att aac gag gaa tca ctg gat agg gct agg aga ttg ctt tgg tgg 576

Lys Ile Asn Glu Glu Ser Leu Asp Arg Ala Arg Arg Leu Leu Trp Trp

180

185

190

cat tac aac tgt tta ctg tgg gga gaa gct cca gtt act aac tat att 624

His Tyr Asn Cys Leu Leu Trp Gly Glu Ala Gln Val Thr Asn Tyr Ile

195

200

205

tct cgt ttg cgt act tgg ttg tca act cct gag aaa tat aga ggt aga 672

Ser Arg Leu Arg Thr Trp Leu Ser Thr Pro Glu Lys Tyr Arg Gly Arg

210

215

220

gat gcc ccg acc att gaa gca atc act aga cca atc cag gtg gct cag 720

Asp Ala Pro Thr Ile Glu Ala Ile Thr Arg Pro Ile Gln Val Ala Gln

225

230

235

240

gga ggc aga aaa aca act acg ggt act aga aaa cct cgt gga ctc gaa 768

Gly Gly Arg Lys Thr Thr Gly Thr Arg Lys Pro Arg Gly Leu Glu

245

250

255

cct aga aga aga aaa gtt aaa acc aca gtt gtc tat ggg aga aga cgt 816

Pro Arg Arg Arg Lys Val Lys Thr Thr Val Val Tyr Gly Arg Arg Arg

260

265

270

tca aag tcc cgg gaa agg aga gcc cct aca ccc caa cgt gcg ggc tcc 864

Ser Lys Ser Arg Glu Arg Arg Ala Pro Thr Pro Gln Arg Ala Gly Ser

275

280

285

cct ctc cca cgt agt tcg agc agc cac cat aga tct ccc tcg cct agg 912

Pro Leu Pro Arg Ser Ser Ser Ser His His Arg Ser Pro Ser Pro Arg

290

295

300

aaa taa

918

Lys

305

WO 99/40934

PCT/US99/03055

<211> 305

<212> PRT

<213> Hepatitis B virus

<400> 13

Met Trp Asn Leu Arg Ile Thr Pro Leu Ser Phe Gly Ala Ala Cys Gln
1 5 10 15

Gly Ile Phe Thr Ser Thr Leu Leu Leu Ser Cys Val Thr Val Pro Leu
20 25 30

Val Cys Thr Ile Val Tyr Asp Ser Cys Leu Tyr Met Asp Ile Asn Ala
35 40 45

Ser Arg Ala Leu Ala Asn Val Tyr Asp Leu Pro Asp Asp Phe Phe Pro
50 55 60

Lys Ile Asp Asp Leu Val Arg Asp Ala Lys Asp Ala Leu Glu Pro Tyr
65 70 75 80

Trp Lys Ser Asp Ser Ile Lys Lys His Val Leu Ile Ala Thr His Phe
85 90 95

Val Asp Leu Ile Glu Asp Phe Trp Gln Thr Thr Gln Gly Met His Glu
100 105 110

Ile Ala Glu Ser Leu Arg Ala Val Ile Pro Pro Thr Thr Thr Pro Val
115 120 125

Pro Pro Gly Tyr Leu Ile Gln His Glu Glu Ala Glu Glu Ile Pro Leu
130 135 140

Gly Asp Leu Phe Lys His Gln Glu Glu Arg Ile Val Ser Phe Gln Pro
145 150 155 160

Asp Tyr Pro Ile Thr Ala Arg Ile His Ala His Leu Lys Ala Tyr Ala
165 170 175

Lys Ile Asn Glu Glu Ser Leu Asp Arg Ala Arg Arg Leu Leu Trp Trp
180 185 190

His Tyr Asn Cys Leu Leu Trp Gly Glu Ala Gln Val Thr Asn Tyr Ile
195 200 205

Ser Arg Leu Arg Thr Trp Leu Ser Thr Pro Glu Lys Tyr Arg Gly Arg
210 215 220

Asp Ala Pro Thr Ile Glu Ala Ile Thr Arg Pro Ile Gln Val Ala Gln

WO 99/40934

PCT/US99/03055

225

230

235

240

Gly Gly Arg Lys Thr Thr Thr Gly Thr Arg Lys Pro Arg Gly Leu Glu
 245 250 255

Pro Arg Arg Arg Lys Val Lys Thr Thr Val Val Tyr Gly Arg Arg Arg
 260 265 270

Ser Lys Ser Arg Glu Arg Arg Ala Pro Thr Pro Gln Arg Ala Gly Ser
 275 280 285

Pro Leu Pro Arg Ser Ser Ser Ser His His Arg Ser Pro Ser Pro Arg
 290 295 300

Lys

305

<210> 14

<211> 37

<212> PRT

<213> Influenza virus

<400> 14

Ser Ile Met Arg Ser Asp Ala Pro Ile Gly Thr Cys Ser Ser Glu Cys
 1 5 10 15

Ile Thr Pro Asn Gly Ser Ile Pro Asn Asp Lys Pro Phe Gln Asn Val
 20 25 30

Asn Lys Ile Thr Tyr

35

<210> 15

<211> 29

<212> PRT

<213> Influenza virus

<400> 15

Arg Gly Ile Phe Gly Ala Ile Ala Gly Phe Ile Glu Asn Gly Trp Glu
 1 5 10 15

Gly Met Ile Asp Gly Trp Tyr Gly Phe Arg His Gln Asn
 20 25

<210> 16

WO 99/40934

PCT/US99/03055

<211> 10

<212> PRT

<213> Influenza virus

<400> 16

Glu Lys Gln Thr Arg Gly Ile Phe Gly Ala

1

5

10

<210> 17

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Hepatitis B
virus PCR primer with an NcoI restriction site.

<400> 17

ttgggccatg gacatcgacc tta

23

<210> 18

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Hepatitis B
virus PCR primer with an EcoRI restriction site

<400> 18

gcggaattcc atcttccaaa ttaacaccca c

31

<210> 19

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Hepatitis B
virus PCR primer with EcoRI and SacI restriction
sites and an inserted lysine codon

<400> 19

cgcgaattca aaaagagctc ccagcgatca gagagaccta g

41

WO 99/40934

PCT/US99/03055

<210> 20

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Hepatitis B
virus PCR primer with HindIII restriction site

<400> 20

cgcaagctta aacaacagta gtctccggaa g

31

<210> 21

<211> 14

<212> PRT

<213> Hepatitis B virus

<400> 21

Cys Gln Glu Lys Gln Leu Asp Glu Asn Ala Asn Val Gln Leu

1

5

10

<210> 22

<211> 13

<212> PRT

<213> Hepatitis B virus

<400> 22

Cys Ser Lys Lys Gly Pro Arg Ala Ser Gly Asn Leu Ile

1

5

10

<210> 23

<211> 19

<212> PRT

<213> Hepatitis B virus

<400> 23

Cys Leu Thr Glu His Arg Met Thr Trp Asp Pro Ala Gln Pro Pro Arg

1

5

10

15

Asp Leu Thr

<210> 24

WO 99/40934

PCT/US99/03055

<211> 22

<212> PRT

<213> Hepatitis B virus

<400> 24

Cys Val Lys Arg Met Lys Glu Ser Arg Leu Glu Asp Thr Gln Lys His
1 5 10 15

Arg Val Asp Phe Leu Gln

20

<210> 25

<211> 15

<212> PRT

<213> Streptococcus pneumoniae

<300>

<310> EP-O 786 521-A

<400> 25

Lys Leu Glu Glu Leu Ser Asp Lys Ile Asp Glu Leu Asp Ala Glu
1 5 10 15

<210> 26

<211> 35

<212> PRT

<213> Streptococcus pneumoniae

<300>

<310> EP-O 786 521-A

<400> 26

Gln Lys Lys Tyr Asp Glu Asp Gln Lys Lys Thr Glu Glu Lys Ala Ala
1 5 10 15

Leu Glu Lys Ala Ala Ser Glu Glu Met Asp Lys Ala Val Ala Ala Val
20 25 30

Gln Gln Ala

35

<210> 27

<211> 31

<212> PRT

<213> Cryptosporidium parvum

WO 99/40934

PCT/US99/03055

<300>

<310> WO 98/07320

<400> 27

Gln Asp Lys Pro Ala Asp Ala Pro Ala Ala Glu Ala Pro Ala Ala Glu
1 5 10 15

Pro Ala Ala Glu Pro Ala Ala Gln Asp Lys Pro Ala Asp Ala
20 25 30

<210> 28

<211> 16

<212> PRT

<213> Human immunodeficiency virus

<300>

<310> US 5,639,854

<400> 28

Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Cys
1 5 10 15

<210> 29

<211> 17

<212> PRT

<213> Human immunodeficiency virus

<300>

<310> WO 98/07320

<400> 29

Arg Lys Arg Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Ile Thr Lys
1 5 10 15

Asn

<210> 30

<211> 31

<212> PRT

<213> Foot-and-mouth disease virus

<300>

<310> US 4,544,500

WO 99/40934

PCT/US99/03055

<211> 1.

<212> PRT

<213> *Borrelia burgdorferi*

<300>

<301> Zhong, W.

et al.,

<303> *Eur. J. Immunol.*

<304> 26

<305> 11

<306> 2749-

<307> 1996

<400> 33

Thr Leu Ser Lys Asn Ile Ser Lys Ser Gly Glu Val Ser Val Glu Leu

1

5

10

15

Asn Asp Cys

<210> 34

<211> 11

<212> PRT

<213> *Influenza A virus*

<300>

<301> Brumeau, T. D.

<303> *Immunotechnology*

<304> 2

<305> 2

<306> 85-

<307> (1996)

<400> 34

Ser Ser Val Ser Ser Phe Glu Arg Phe Glu Cys

1

5

10

<210> 35

<211> 10

<212> PRT

<213> *Influenza A virus*

<300>

<301> Brumeau, T. D.

<303> *Immunotechnology*

<304> 2

WO 99/40934

PCT/US99/03055

<305> 2

<306> 85-

<307> (1996)

<400> 35

Tyr Arg Asn Leu Leu Trp Leu Thr Glu Lys
 1 5 10

<210> 36

<211> 142

<212> PRT

<213> Yersinia pestis

<300>

<301> Hill, J.

et al.,

<303> Infect. Immun.

<304> 65

<305> 11

<306> 4476-

<307> (1997)

<400> 36

Asp Ile Leu Lys Val Ile Val Asp Ser Met Asn His His Gly Asp Ala
 1 5 10 15

Arg Ser Lys Leu Arg Glu Glu Leu Ala Glu Leu Thr Ala Glu Leu Lys
 20 25 30

Ile Tyr Ser Val Ile Gln Ala Glu Ile Asn Lys His Leu Ser Ser Ser
 35 40 45

Gly Thr Ile Asn Ile-His Asp Lys Ser Ile Asn Leu Met Asp Lys Asn
 50 55 60

Leu Tyr Gly Tyr Thr Asp Glu Glu Ile Phe Lys Ala Ser Ala Glu Tyr
 65 70 75 80

Lys Ile Leu Glu Lys Met Pro Gln Thr Thr Ile Gln Val Asp Gly Ser
 85 90 95

Glu Lys Lys Ile Val Ser Ile Lys Asp Phe Leu Gly Ser Glu Asn Lys
 100 105 110

Arg Thr Gly Ala Leu Gly Asn Leu Lys Asn Ser Tyr Ser Tyr Asn Lys
 115 120 125

Asp Asn Asn Glu Leu Ser His Phe Ala Thr Thr Cys Ser Asp
130 135 140

<210> 37

<211> 19

<212> PRT

<213> Haemophilus influenzae

<300>

<310> EP-O 432 220-B1

<400> 37

Cys Ser Ser Ser Asn Asn Asp Ala Ala Gly Asn Gly Ala Ala Gln Phe
1 5 10 15

Gly Gly Tyr

<210> 38

<211> 11

<212> PRT

<213> Haemophilus influenzae

<300>

<310> EP-O 432 220-B1

<400> 38

Asn Lys Leu Gly Thr Val Ser Tyr Gly Glu Glu
1 5 10

<210> 39

<211> 16

<212> PRT

<213> Haemophilus influenzae

<300>

<310> EP-O 432 220-B1

<400> 39

Asn Asp Glu Ala Ala Tyr Ser Lys Asn Arg Arg Ala Val Leu Ala Tyr
1 5 10 15

<210> 40

<211> 28

WO 99/40934

PCT/US99/03055

<212> PRT

<213> Moraxella catarrhalis

<300>

<310> WO 98/06851

<400> 40

Leu Asp Ile Glu Lys Asp Lys Lys Arg Thr Asp Glu Gln Leu Gln

1

5

10

15

Ala Glu Leu Asp Asp Lys Tyr Ala Gly Lys Gly Tyr

20

25

<210> 41

<211> 28

<212> PRT

<213> Moraxella catarrhalis

<300>

<310> WO 98/06851

<400> 41

Leu Asp Ile Glu Lys Asn Lys Lys Arg Thr Glu Ala Glu Leu Gln

1

5

10

15

Ala Glu Leu Asp Asp Lys Tyr Ala Gly Lys Gly Tyr

20

25

<210> 42

<211> 28

<212> PRT

<213> Moraxella catarrhalis

<300>

<310> WO 98/06851

<400> 42

Ile Asp Ile Glu Lys Lys Gly Lys Ile Arg Thr Glu Ala Glu Leu Leu

1

5

10

15

Ala Glu Leu Asn Lys Asp Tyr Pro Gly Gln Gly Tyr

20

25

<210> 43

<211> 25

WO 99/40934

PCT/US99/03055

<212> PRT

<213> Porphyromonas gingivalis

<300>

<304> 110

<305> 2

<306> 285-

<307> 1997

<400> 43

Gly Val Ser Pro Lys Val Cys Lys Asp Val Thr Val Glu Gly Ser Asn
1 5 10 15

Glu Phe Ala Pro Val Gln Asn Leu Thr
20 25

<210> 44

<211> 20

<212> PRT

<213> Porphyromonas gingivalis

<300>

<304> 110

<305> 2

<306> 285-

<307> 1997

<400> 44

Arg Ile Gln Ser Thr Trp Arg Gln Lys Thr Val Asp Leu Pro Ala Gly
1 5 10 15

Thr Lys Tyr Val
20

<210> 45

<211> 20

<212> PRT

<213> Trypanosoma cruzi

<300>

<304> 159

<305> 9

<306> 4444-

<307> (1997)

<400> 45

WO 99/40934

PCT/US99/03055

Ser His Asn Phe Thr Leu Val Ala Ser Val Ile Ile Glu Ala Pro Ser

1

5

10

15

Gly Asn Thr Cys

20

<210> 46

<211> 21

<212> PRT

<213> Trypanosoma cruzi

<300>

<310> WO 97/18475

<400> 46

Lys Ala Ala Ile Ala Pro Ala Lys Ala Ala Ala Ala Pro Ala Lys Ala

1

5

10

15

Ala Thr Ala Pro Ala

20

<210> 47

<211> 16

<212> PRT

<213> Plasmodium falciparum

<300>

<303> Int. Arch. Allergy Appl. Immunol.

<304> 114

<305> 1

<306> 15-

<400> 47

Ser Val Gln Ile Pro Lys Val Pro Tyr Pro Asn Gly Ile Val Tyr Cys

1

5

10

15

<210> 48

<211> 16

<212> PRT

<213> Plasmodium falciparum

<30 >

<303> Int. Arch. Allergy Appl. Immunol.

<304> 114

<305> 1

WO 99/40934

PCT/US99/03055

<306> 15-

<400> 48

Asp Phe Asn His Tyr Tyr Thr Leu Lys Thr Gly Leu Glu Ala Asp Cys
1 5 10 15

<210> 49

<211> 16

<212> PRT

<213> *Streptococcus sobrinus*

<300>

<303> Arch. Oral Biol.

<304> 35

<306> Suppl. 475-

<307> (1990)

<400> 49

Lys Pro Arg Pro Ile Tyr Glu Ala Lys Leu Ala Gln Asn Gln Lys Cys
1 5 10 15

<210> 50

<211> 17

<212> PRT

<213> *Streptococcus sobrinus*

<300>

<303> Arch. Oral Biol.

<304> 35

<306> Suppl. 475-

<307> (1990)

<400> 50

Ala Lys Ala Asp Tyr Glu Ala Lys Leu Ala Gln Tyr Glu Lys Asp Leu
1 5 10 15

Cys

<210> 51

<211> 16

<212> PRT

<213> *Lymphocytic choriomeningitis virus*

<300>

WO 99/40934

PCT/US99/03055

<303> Proc. Natl. Acad. Sci. U.S.A.

<304> 94

<305> 7

<306> 3314-

<307> (1997)

<400> 51

Arg Pro Gln Ala Ser Gly Val Tyr Met Gly Asn Leu Thr Ala Gln Cys

1

5

10

15

<210> 52

<211> 9

<212> PRT

<213> Shigella flexneri

<300>

<303> J. Biol. Chem.

<304> 271

<305> 52

<306> 33670-

<307> (1996)

<400> 52

Lys Asp Arg Thr Leu Ile Glu Gln Lys

1

5

<210> 53

<211> 15

<212> PRT

<213> respiratory syncytial virus

<300>

<304> 234

<305> 1

<306> 118-

<307> 1997

<400> 53

Cys Ser Ile Cys Ser Asn Asn Pro Thr Cys Trp Ala Ile Cys Lys

1

5

10

15

<210> 54

<211> 19

<212> PRT

<213> Plasmodium vivax

WO 99/40934

PCT/US99/03055

<300>
 <303> Vaccine
 <304> 15
 <305> 4
 <306> 377-
 <307> 1997

<400> 54

Gly	Asp	Arg	Ala	Asp	Gly	Gln	Pro	Ala	Gly	Asp	Arg	Ala	Asp	Gly	Gln		
1							5							10			15

Pro Ala Gly

<210> 55
 <211> 16
 <212> PRT
 <213> Clostridium tetani

<300>
 <303> Vaccine
 <304> 15
 <305> 4
 <306> 377-
 <307> 1997

<400> 55

Gln	Tyr	Ile	Lys	Ala	Asn	Ser	Lys	Phe	Ile	Gly	Ile	Thr	Glu	Leu	Cys	
1									5					10		15

<210> 56
 <211> 25
 <212> PRT
 <213> Entamoeba histolytica

<300>
 <303> J. Exp. Med.
 <304> 185
 <305> 10
 <306> 1793-
 <307> 1997

<400> 56

Val	Glu	Cys	Ala	Ser	Thr	Val	Cys	Gln	Asn	Asp	Asn	Ser	Cys	Pro	Ile	
1									5					10		15

WO 99/40934

PCT/US99/03055

Ile Ala Asp Val Glu Lys Cys Asn Gln
20 25

<210> 57
<211> 34
<212> PRT
<213> Schistosoma japonicum

<300>
<303> Vaccine
<304> 15
<305> 1
<306> 79-
<307> 1997

<400> 57
Asp Leu Gln Ser Glu Ile Ser Leu Ser Leu Glu Asn Gly Glu Leu Ile
1 5 10 15

Arg Arg Ala Lys Ser Ala Glu Ser Leu Ala Ser Glu Leu Gln Arg Arg
20 25 30

Val Asp

<210> 58
<211> 34
<212> PRT
<213> Schistosoma mansoni

<300>
<303> Vaccine
<304> 15
<305> 1
<306> 79-
<307> 1997

<400> 58
Asp Leu Gln Ser Glu Ile Ser Leu Ser Leu Glu Asn Ser Glu Leu Ile
1 5 10 15

Arg Arg Ala Lys Ala Ala Glu Ser Leu Ala Ser Asp Leu Gln Arg Arg
20 25 30

Val Asp

WO 99/40934

PCT/US99/03055

<210> 59

<211> 63

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: MseI
restriction endonuclease site inserted into wild
type Hepatitis B sequence at position 75

<220>

<221> CDS

<222> (1)..(63)

<400> 59

gct acc tgg gtg ggt gtt aat ttg gaa gat cca gcg tct aga gac cta 48
Ala Thr Trp Val Gly Val Asn Leu Glu Asp Pro Ala Ser Arg Asp Leu
1 5 10 15

gta gtc agt tat gtc
Val Val Ser Tyr Val
20

63

<210> 60

<211> 21

<212> PRT

<213> Artificial Sequence

<400> 60

Ala Thr Trp Val Gly Val Asn Leu Glu Asp Pro Ala Ser Arg Asp Leu
1 5 10 15

Val Val Ser Tyr Val
20

63

<210> 61

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: K inserted at
amino acid position 75 of Hepatitis B core

sequence

<220>

<221> CDS

<222> (1)..(39)

<400> 61

gct acc tgg gtg ggt gtt aaa aat ttg gaa gat cca gcg tc
Ala Thr Trp Val Gly Val Lys Asn Leu Glu Asp Pro Ala

41

1

5

10

<210> 62

<211> 13

<212> PRT

<213> Artificial Sequence

<400> 62

Ala Thr Trp Val Gly Val Lys Asn Leu Glu Asp Pro Ala

1

5

10

<210> 63

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: K inserted at
amino acid position 76 of hepatitis B core

<220>

<221> CDS

<222> (3)..(26)

<400> 63

tt aat aaa ttg ga~~gg~~at cca gcg tct a
Asn Lys Leu Glu Asp Pro Ala Ser

27

1

5

<210> 64

<211> 8

<212> PRT

<213> Artificial S quence

<400> 64

Asn Lys Leu Glu Asp Pro Ala Ser

WO 99/40934

PCT/US99/03055

1

5

<210> 65

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: K inserted at
position 77 of hepatitis B virus core

<220>

<221> CDS

<222> (3)...(26)

<400> 65

tt aat ttg aaa gaa gat cca gcg tct a
Asn Leu Lys Glu Asp Pro Ala Ser

1

5

27

<210> 66

<211> 8

<212> PRT

<213> Artificial Sequence

<400> 66

Asn Leu Lys Glu Asp Pro Ala Ser

1

5

<210> 67

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: K inserted at
position 78 of hepatitis B core

<220>

<221> CDS

<222> (3)...(32)

<400> 67

tt aat ttg gaa aaa gat cca gcg tct aga gac
Asn Leu Glu Asp Pro Ala Ser Arg Asp

32

WO 99/40934

PCT/US99/03055

1

5

10

<210> 68

<211> 10

<212> PRT

<213> Artificial Sequence

<400> 68

Asn Leu Glu Lys Asp Pro Ala Ser Arg Asp

1

5

10

<210> 69

<211> 36

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: K inserted at
amino acid position 79 of hepatitis B core

<220>

<221> CDS

<222> (3)..(35)

<400> 69

tt aat ttg gaa gat aaa cca gcg tct aga gac cta g

36

Asn Leu Glu Asp Lys Pro Ala Ser Arg Asp Leu

1

5

10

<210> 70

<211> 11

<212> PRT

<213> Artificial Sequence

<400> 70

Asn Leu Glu Asp Lys Pro Ala Ser Arg Asp Leu

1

5

10

<210> 71

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

WO 99/40934

PCT/US99/03055

<223> Description of Artificial Sequence: K inserted at amino acid position 80 of hepatitis B core

<220>

<221> CDS

<222> (3) .. (38)

<400> 71

tt aat ttg gaa gat cca aaa gcg tct aga gac cta gta g
Asn Leu Glu Asp Pro Lys Ala Ser Arg Asp Leu Val

1 5 10

39

<210> 72

<211> 12

<212> PRT

<213> Artificial Sequence

<400> 72

Asn Leu Glu Asp Pro Lys Ala Ser Arg Asp Leu Val

1 5 10

<210> 73

<211> 43

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: K inserted at amino acid position 81 of hepatitis B core

<220>

<221> CDS

<222> (3) .. (41)

<400> 73

tt aat ttg gaa gat cca gcg aaa tct aga gac cta gta gtc ag
Asn Leu Glu Asp Pro Ala Lys Ser Arg Asp Leu Val

1 5 10

43

<210> 74

<211> 13

<212> PRT

<213> Artificial Sequence

<400> 74

Asn Leu Glu Asp Pro Ala Lys Ser Arg Asp Leu Val Val

1

5

10

<210> 75

<211> 45

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: K inserted at
amino acid position 82 of hepatitis B core

<220>

<221> CDS

<222> (3)..(44)

<400> 75

tt aat ttg gaa gat cca gcg tct aaa aga gac cta gta gtc agt t
Asn Leu Glu Asp Pro Ala Ser Lys Arg Asp Leu Val Val Ser

1

5

10

45

<210> 76

<211> 14

<212> PRT

<213> Artificial Sequence

<400> 76

Asn Leu Glu Asp Pro Ala Ser Lys Arg Asp Leu Val Val Ser
1 5 10

<210> 77

<211> 50

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: K inserted at
amino acid position 83 of hepatitis B core

<220>

<221> CDS

<222> (3)..(50)

<400> 77

tt aat ttg gaa gat cca gcg tct aga aaa gac cta gta gtc agt tat 47

WO 99/40934

PCT/US99/03055

Asn Leu Glu Asp Pro Ala Ser Arg Lys Asp Leu Val Val Ser Tyr
 1 5 10 15

gtc 50
 Val

<210> 78

<211> 16

<212> PRT

<213> Artificial Sequence

<400> 78

Asn Leu Glu Asp Pro Ala Ser Arg Lys Asp Leu Val Val Ser Tyr Val
 1 5 10 15

<210> 79

<211> 50

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: K inserted at
 amino acid position 84 of hepatitis B core

<220>

<221> CDS

<222> (3)..(50)

<400> 79

ttaat ttg gaa gat cca gcg tct aga gac aaa cta gta gtc agt tat 47
 Asn Leu Glu Asp Pro Ala Ser Arg Asp Lys Leu Val Val Ser Tyr
 1 5 10 15

gtc 50
 Val

<210> 80

<211> 16

<212> PRT

<213> Artificial Sequence

<400> 80

Asn Leu Glu Asp Pro Ala Ser Arg Asp Lys Leu Val Val Ser Tyr Val
 1 5 10 15

WO 99/40934

PCT/US99/03055

<210> 81
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: K inserted at amino acid position 85 of hepatitis B core

<220>
<221> CDS
<222> (2)..(31)

<400> 81
c tcg aga gac cta aaa gta gtc agt tat gtc
Ser Arg Asp Leu Lys Val Val Ser Tyr Val
1 5 10

31

<210> 82
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 82
Ser Arg Asp Leu Lys Val Val Ser Tyr Val
1 5 10

<210> 83
<211> 18
<212> PRT
<213> Plasmodium vivax

<400> 83
Asp Arg Ala Ala Gly Gln Pro Ala Gly Asp Arg Ala Asp Gly Gln Pro
1 5 10 15

Ala Gly

<210> 84
<211> 8
<212> PRT
<213> Influenza virus

WO 99/40934

PCT/US99/03055

<400> 84

Cys Asn Asn Pro His Arg Ile Leu

1 5

<210> 85

<211> 25

<212> PRT

<213> Influenza virus

<400> 85

Cys Pro Lys Tyr Val Lys Gln Asn Thr Leu Lys Leu Ala Thr Gly Met

1 5 10 15

Arg Asn Val Pro Glu Lys Gln Thr Arg

20 25

<210> 86

<211> 15

<212> PRT

<213> Mycobacterium tuberculosis

<400> 86

Ala Val Leu Glu Asp Pro Tyr Ile Leu Leu Val Ser Ser Lys Val

1 5 10 15.

<210> 87

<211> 15

<212> PRT

<213> Mycobacterium tuberculosis

<400> 87

Leu Leu Val Ser Ser Lys Val Ser Thr Val Lys Asp Leu Leu Pro

1 5 10 15

<210> 88

<211> 15

<212> PRT

<213> Mycobacterium tuberculosis

<400> 88

Leu Leu Pro Leu Leu Glu Val Ile Gly Ala Gly Lys Pro Leu

1 5 10 15

WO 99/40934

PCT/US99/03055

<210> 89

<211> 15

<212> PRT

<213> Mycobacterium tuberculosis

<400> 89

Ala Ile Leu Thr Gly Gly Gln Val Ile Ser Glu Glu Val Gly Leu
1 5 10 15

<210> 90

<211> 15

<212> PRT

<213> Mycobacterium tuberculosis

<400> 90

Ile Ala Phe Asn Ser Gly Leu Glu Pro Gly Val Val Ala Glu Lys
1 5 10 15

<210> 91

<211> 18

<212> PRT

<213> Mycobacterium tuberculosis

<400> 91

Ala Arg Arg Gly Leu Glu Arg Gly Leu Asn Ala Leu Ala Asp Ala Val
1 5 10 15

Lys Val

<210> 92

<211> 14

<212> PRT

<213> Mycobacterium tuberculosis

<400> 92

Glu Lys Ile Gly Ala Glu Leu Val Lys Glu Val Ala Lys Lys
1 5 10

<210> 93

<211> 17

<212> PRT

<213> Mycobacterium tuberculosis

WO 99/40934

PCT/US99/03055

<400> 93

Gly Leu Lys Arg Gly Ile Glu Lys Ala Val Glu Lys Val Thr Glu Thr

1 5 10 15

Leu

<210> 94

<211> 15

<212> PRT

<213> Mycobacterium tuberculosis

<400> 94

Ile Glu Asp Ala Val Arg Asn Ala Lys Ala Ala Val Glu Glu Gly

1 5 10 15

<210> 95

<211> 20

<212> PRT

<213> Feline leukemia virus

<400> 95

Cys Asp Ile Ile Gly Asn Thr Trp Asn Pro Ser Asp Gln Glu Pro Phe

1 5 10 15

Pro Gly Tyr Gly

20

<210> 96

<211> 21

<212> PRT

<213> Feline leukemia virus

<400> 96

Cys Ile Gly Thr Val Pro Lys Thr His Gln Ala Leu Cys Asn Glu Thr

1 5 10 15

Gln Gln Gly His Thr

20

<210> 97

<211> 13

<212> PRT

<213> Feline leukemia virus

WO 99/40934

PCT/US99/03055

<400> 97

Gly Asn Tyr Ser Asn Gln Thr Asn Pro Pro Pro Ser Cys
1 5 10

<210> 98

<211> 22

<212> PRT

<213> Feline leukemia virus

<400> 98

Thr Asp Ile Gln Ala Leu Glu Glu Ser Ile Ser Ala Leu Glu Lys Ser
1 5 10 15

Leu Thr Ser Leu Ser Glu

20

<210> 99

<211> 14

<212> PRT

<213> Feline leukemia virus

<400> 99

Ala Lys Leu Arg Glu Arg Leu Lys Gln Arg Gln Gln Leu Phe
1 5 10

<210> 100

<211> 23

<212> PRT

<213> Feline leukemia virus

<400> 100

Asp Ser Gln Gln Gly Trp Phe Glu Gly Trp Phe Asn Lys Ser Pro Trp
1 5 10 15

Phe Thr Thr Leu Ile Ser Ser

20

<210> 101

<211> 18

<212> PRT

<213> Feline leukemia virus

<400> 101

WO 99/40934

PCT/US99/03055

Gln Val Met Thr I1 Thr Pro Pro Gln Ala Met ly Pro Asn Leu Val
 1 5 10 15

Leu Pro

<210> 102

<211> 17

<212> PRT

<213> Feline leukemia virus

<400> 102

Asp Gln Lys Pro Pro Ser Arg Gln Ser Gln Ile Glu Ser Arg Val Thr
 1 5 10 15

Pro

<210> 103

<211> 15

<212> PRT

<213> Feline leukemia virus

<400> 103

Arg Arg Gly Leu Asp Ile Leu Phe Leu Gln Glu Gly Gly Leu Cys
 1 5 10 15

<210> 104

<211> 25

<212> PRT

<213> Feline leukemia virus

<400> 104

Gln Glu Gly Gly Leu Cys Ala Ala Leu Glu Glu Cys Gln Ile Gly Gly
 1 5 10 15

Leu Cys Ala Ala Leu Lys Glu Glu Cys
 20 25

<210> 105

<211> 12

<212> PRT

<213> Artificial Sequence

WO 99/40934

PCT/US99/03055

<220> Description of Artificial Sequence: Cytochrome P-450 fragment

<300>
<303> Science
<304> 228
<306> 1436-1440
<307> 1985

<400> 105
Asn Ala Asn Pro Asn Ala Asn Pro Asn Ala Asn Pro
1 5 10

<210> 106
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Cytochrome
P-450 fragment

<400> 106
Cys Met Gln Leu Arg Ser
1 5

<210> 107
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Cytochrome
P-450 fragment

<400> 107
Cys Arg Phe Ser Ile Asn
1 5

<210> 108
<211> 5
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Cytochrome
P-450 fragment

<400> 108

Cys Ala Val Pro Arg

1 5

<210> 109

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Cytochrome
P-450 fragment

<400> 109

Cys Ile Pro Arg Ser

1 5

<210> 110

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Cytochrome
P-450 fragment.

<400> 110

Cys Phe Ile Pro Val

1 5

<210> 111

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Cytochrome
P-450 fragment

<400> 111

Cys Thr Val Ser Gly Ala

WO 99/40934

PCT/US99/03055

1 5

<210> 112
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Cytochrome
P-450 fragment

<400> 112
Cys Thr Leu Ser Gly Glu
1 5

<210> 113
<211> 12
<212> PRT
<213> Plasmodium knowlesi

<400> 113
Gln Ala Gln Gly Asp Gly Ala Asn Ala Gly Gln Pro
 1 5 10

INTERNATIONAL SEARCH REPORT

International application N
PCT/US99/03055

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K39/00, 39/38, 39/29; C07K 7/00, 15/00

US CL : 424/184.1, 189.1; 530/324, 325, 326; 536/23.72

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/184.1, 189.1; 530/324, 325, 326; 536/23.72

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, APS, CAS ONLINE

search terms: hepatitis B, core protein, hapten, fusio?, pendent?, antigenic, Ab, immunoglo?, antibodies.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KOLETZKI et al. Mosaic hepatitis B virus core particles allow insertion of extended foreign protein segments. Journal of General Virology. 1997, Vol. 78, pages 2049-2053, see the entire document.	1-22
Y	SHIAU et al. Mutated Epitopes of Hepatitis B Surface Antigen Fused to the Core Antigen of the Virus Induce Antibodies That React With the Native Surface Antigen. Journal of Medical Virology. 1997, Vol. 51, pages 159-166, see the entire document.	1-22
Y	US 5,143,726 A (THORNTON et al) 01 September 1992, see the claims.	1-22
Y	US 4,818,527 A (THORNTON et al) 04 April 1989, see the claims	1-22

 Further documents are listed in the continuation of Box C.

See patent family annex.

Special categories of cited documents:	
A	document defining the general state of the art which is not considered to be of particular relevance
B	earlier document published on or after the international filing date
L	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
O	document referring to an oral disclosure, use, exhibition or other means
P	document published prior to the international filing date but later than the priority date claimed
T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
A	document not member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

20 MAY 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Authorized officer

Telephone No.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/03055

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,882,145 A (THORNTON et al) 21 November 1989, see the claims.	1-22
A.E	US 5,840,303 A (CHISARI et al) 24 November 1998, see the abstract, and the claims.	1-22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/03055

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/03055

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

Group I, claim(s) 1-7, drawn to hepatitis B core protein conjugated to a hapten wherein hapten is pendently linked wherein hepatitis protein is identified with a specific sequence.

Group II, claim(s) 8, drawn to hepatitis B core protein particles comprising an insert that is antigenic.

Group III, claim(s) 9-13, drawn to hepatitis B core protein pendently linked to a hapten.

Group IV, claim(s) 14, 15, drawn to an immunogenic fusion protein conjugated to hapten pendently linked to three peptide domains of hepatitis B core protein.

Group V, claim(s) 16, drawn to hepatitis B core protein particles comprising an insert that is non-antigenic.

Group VI, claim(s) 17-19, drawn to a vaccine composition of hepatitis B core protein and a hapten.

Group VII, claim(s) 20-22, drawn to process of inducing antibodies against hepatitis B core protein and hapten.

The inventions listed as Groups I-VII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Groups I and II-VII are directed to different sets of hepatitis B core proteins with different hapten(s) and multitude of different characteristics, different sets of nucleotide and domains, that are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept, accordingly, the unity of invention is lacking among the groups.